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CONTENTS

FIRST PART—OCTOBER 1939

PAGE

Some observations on the morphology of the synapse in the cat's spinal cord. By MURRAY L. BARR 1

An experimental investigation of the motor cortex and its connexions in the phalanger, *Trichosurus vulpecula*. By F. GOLDBY . . 12

Experiments on the development of the amphibian mesonephros. By R. J. O'CONNOR 34

Some observations on the lymphatics of the nasal mucous membrane in the cat and monkey. By JOSEPH M. YOFFEY and CECIL K. DRINKER 45

The innervation of the gum of *Talpa europea*. By W. LEWINSKY and D. STEWART 53

The cervical region of the Lacertilia. A critical review of certain aspects of its anatomy. By W. E. ADAMS. 57

The occurrence of the lemurine form of the ectotympanic in a primitive marsupial. By F. WOOD JONES and V. F. LAMBERT . . . 72

Radiographic studies of the metacarpal and metatarsal bones in children. By K. POSENER, E. WALKER and GRAHAM WEDDELL . 76

The structure of the epiphyses in *Sphenodon* and the primitive form of secondary centre. By R. WHEELER HAINES 80

The cell structure of the mamillary body in the mammals and in man. By JERZY ROSE 91

Anastomoses between the rectal and uterine veins forming a connexion between the somatic and portal venous system in the recto-uterine pouch. By E. G. WERMUTH 116

ANATOMICAL NOTES: A modification of the technique of wax plate construction. By C. S. HALLPIKE and E. W. PEET . . . 127

The presence of a fronto-palatine articulation in Aboriginal Australian skulls. By K. F. RUSSELL 129

Pericardio-peritoneal communication. Description of a rare type of diaphragmatic hernia. By H. D. O'BRIEN 131

PROCEEDINGS 137

REVIEWS. A synopsis of regional anatomy. By T. B. JOHNSTON . . 144

The tissues of the body. By W. E. LE GROS CLARK . . 144

Synopsis of surgical anatomy. By A. L. McGREGOR . . 146

A manual of practical anatomy. Part II. The thorax and abdomen. By THOMAS WALMSLEY 146

Handbuch d. vergl. Anatomie d. Wirbeltiere . . . 146

SECOND PART—JANUARY 1940

PAGE

The nature of the soft palate. By FREDERIC WOOD JONES . . . 147

An experimental study of the morphogenesis of intersexuality. By S. ZUCKERMAN and J. R. GROOME. 171

The projection of the cerebral cortex on the pons and cerebellum in the macaque monkey. By S. SUNDERLAND. 201

On the relative position of the hippocampus and the corpus callosum in placental mammals. By F. GOLDBY 227

Corticofugal degeneration following thermocoagulation of areas 4, 6 and 4-s in *Macaca mulatta*. By W. J. C. VERHAART, M.D. and MARGARET A. KENNARD, M.D. 239

The innervation of the musculature of the tongue. By GRAHAM WEDDELL, J. A. HARPMAN, D. G. LAMBLEY and L. YOUNG . . 255

The development of nerve-endings in the respiratory muscles of the sheep. By LILIAN M. DICKSON 268

The development of synovial joints. By JAMES WHILLIS . . . 277

The evolution of the Haversian pattern in bone. By G. N. C. CRAWFORD 284

THIRD PART—APRIL 1940

An experimental study of the development of the amphibian cloaca. By R. J. O'CONNOR 301

Studies of the regeneration of lymphatic vessels. By J. H. GRAY . 309

The spinal accessory nerve in the sheep. By G. J. ROMANES . . 336

The intramural nervous system of the small intestine with special reference to the innervation of the inner subdivision of its circular muscle. By PEI-LIN LI 348

The development of the patella. By ROBERT WALMSLEY . . . 360

Transmutation of vertebrae in the lumbosacral region of the human spine. By MATTHEW YOUNG and J. G. HASTINGS INCE . . 369

A radiographic comparison of the male and female pelvis. By MATTHEW YOUNG and J. G. HASTINGS INCE 374

The fibre components of the laryngeal nerves of *Macaca mulatta*. By R. J. BROCKLEHURST and F. H. EDGEWORTH 386

The comparative morphology of the platysma. A comparative study of the sphincter colli profundus and the trachelo-platysma. By G. S. LIGHTOLLER 390

PAGE

The comparative morphology of the M. caninus. By G. S. Lightoller 397

Cephalomelus: a rare tumour in a lamb. By D. V. Davies . . . 403

Anatomical Notes: Notes on the construction of blotting-paper models. By R. L. de C. H. Saunders 406

In Memoriam, Robert William Reid (with portrait). 409

Reviews. Anatomy of the sheep's brain. By E. A. Brigg, D.Sc. . 411

Human histology, a guide for medical students. By E. R. A. Cooper 411

Principles of development. By Paul Weiss . . . 411

The anatomy of the human skeleton. By J. Ernest Frazer, D.Sc., F.R.C.S. 412

FOURTH PART—JULY 1940

Observations on the neurohistological basis of cutaneous pain. By the late H. H. Woollard, Graham Weddell and J. A. Harpman . 413

The connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus. By the late H. H. Woollard and J. A. Harpman 441

Differentiation of pituicytes in the human foetus. By William M. Shanklin 459

Mounting media and the fading of histological preparations. By F. Davies 464

The nervous and vascular relations of the pineal gland. By W. E. Le Gros Clark 471

The paranasal sinuses of the anthropoid apes. By A. J. E. Cave and R. Wheeler Haines 493

A note on the length of the basilar membrane in man and in various mammals. By J. A. Keen 524

Thalamic degeneration induced by temporal lesions in the cat. By W. H. Waller 528

The distribution of disturbances of sweat secretion after extirpation of certain sympathetic cervical ganglia in man. By L. Guttmann . 537

Proceedings 552

Review. The pineal organ. By Reginald J. Gladstone and Cecil P. G. Wakeley 560

Index 563

JOURNAL OF ANATOMY

SOME OBSERVATIONS ON THE MORPHOLOGY OF THE SYNAPSE IN THE CAT'S SPINAL CORD[1]

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INTRODUCTION

Recent trends in neurophysiology have served to interest physiologists anew in the morphology of the synapse.[2] The important advances in recording and interpreting action potentials in the nervous system and in the study of the humoral transmission of nerve impulses demand a parallel broadening of knowledge in the study of synapses. This is particularly true of mammalian forms. While the most important contributions must await the application of more dependable technical methods to the mammalian central nervous system, as Bodian (1937) has done in the goldfish, yet much may still be learned by the careful application of the older methods.

Interest in the end-bulbs of Held-Auerbach or axon terminations on cell body and dendrite surfaces of other neurones has also been stimulated by recent studies on their experimental degeneration. Hoff (1932, 1934 and 1935), Foerster *et al.* (1933), Gibson (1937) and Schimert (1938) have described swelling, granulation and ultimately complete degeneration of end-bulbs following the operative interruption of fibre pathways.

The purpose of this paper is to record the author's observations on axon terminations in the spinal cord of the mature cat in material prepared by a modification of the reduced silver nitrate method of Cajal.

HISTORICAL REVIEW

Several detailed reviews dealing with the history of the subject of synapse morphology are to be found in the literature. Only a brief summary, therefore, is indicated here.

Axon endings in the central nervous system, as represented by terminal expansions on the surfaces of other neurones, have been known since the latter part of the nineteenth century. They were demonstrated in the brain stem of the new-born guinea-pig by Semi Meyer in 1896 with the aid of a vital staining

[1] This paper represents an extract from a portion of a thesis submitted to the Committee on Graduate Studies of the Faculty of Medicine of the University of Western Ontario in partial fulfilment of the requirements for the degree of Master of Science.

[2] The terms axon termination, bouton terminal (Cajal), Endfuss, end-foot, or end-bulb of Held-Auerbach, and simply end-bulb, may be used synonymously and are so used in this paper. All of these terms refer to the small terminal expansions of axon branches applied to the surface of another neurone. The word "synapse" (Sherrington), used more in the physiological sense, includes not only the axon termination but also the small area of neurone surface with which the ending is in contact.

technique. The early descriptions by Held (1897) and Auerbach (1899) received particular attention and these structures came to be known as the Endfüsse of Held-Auerbach. With the introduction of metallic staining methods for nerve tissue, axon terminations were recorded in text and figure by many investigators. Ramon y Cajal (1908), in his classical reply to the criticisms of Apáthy, stated that these structures had been observed by the following workers: Arnold, Retzius, Smirnow, Kolliker, von Lenhossék, van Gehuchten, Held, P. Ramón, Lugaro, Dogiel, and many others. The conception of axons ending in terminal expansions upon the cell bodies and dendrites of other neurones was popularized by Cajal in his extensive writings. His minute descriptions and those of other investigators of his time were prompted largely by the then current controversy of contiguity versus continuity at the synapse.

Bartelmez (1915), Bartelmez & Hoerr (1933) and Bodian (1937) have published detailed descriptions of axon terminations on Mauthner's cell in teleosts. Particular attention was devoted to the large club endings representing collaterals of the eighth nerve on the dendrites of Mauthner's cell. Small end-bulbs were also seen similar to those which are found in the spinal cord of mammals. The observations of these investigators demand attention because of the excellent technical methods employed. Marui (1919) also studied the endings on Mauthner's cell.

Windle & Clark (1928) and Tiegs (1931) published observations on the end-bulbs in the mammalian spinal cord.

A comparative study was attempted by Phalen & Davenport (1937). They succeeded in demonstrating end-bulbs in the spinal cord of twelve animal species including reptilian, avian and mammalian forms. While differing in details of size and shape, the general appearance of the endings was similar throughout the series.

Axon terminations in the form of end-bulbs are not limited to the central nervous system. They have been demonstrated on cells of sympathetic ganglia by Aronson (1886), De Castro (1930) and Lawrentjew (1934).

TECHNICAL METHODS

Preparations were made from the spinal cords of thirty mature cats. In preliminary experiments the various modifications of Cajal's reduced silver nitrate method were tried (Lee, *The Microtomist's Vade-Mecum*). Modification 4, involving formalin fixation, was found to be most useful since this method produced the most complete and constant staining of the finer nerve structures.

The highest proportion of good preparations was obtained by the following procedure:

(1) Under ether anaesthesia the abdomen was opened and the left common iliac and middle sacral arteries and all of the visceral branches of the abdominal aorta were clamped.

(2) 300–500 c.c. of 15 % neutral formalin were injected into the vascular system through a cannula in the right common iliac artery. When blocks were

to be taken only from the lumbar and sacral regions of the cord, the cranial end of the abdominal aorta was clamped and the blood and perfusion fluid allowed to escape from the opened inferior vena cava. If the whole of the cord were to be perfused the axillary arteries were clamped and the blood and perfusion fluid permitted to escape from the inferior vena cava and jugular veins.

(3) The spinal cord was removed immediately and freed from the dura mater. After cutting into small blocks fixation was continued for 12 hr. in 15 % neutral formalin.

(4) Blocks rinsed in frequent changes of distilled water for 8 hr.

(5) The blocks were placed for 24 hr. in 96 % alcohol, to each 100 c.c. of which had been added 10 drops of concentrated ammonia.

(6) Excessive alcohol removed with blotting paper and blocks placed in 1·5 % silver nitrate solution at 38° C. for 48–72 hr. Within these limits the length of time in silver nitrate is not critical.

(7) Rinsed in distilled water for 1–2 min. and transferred to 2 % pyrogallol solution for 24 hr., in the dark and at room temperature.

(8) Blocks dehydrated, cleared, imbedded in paraffin and cut serially at 12 μ.

Useful preparations are more constantly obtained by using double distilled water, redistilled alcohol, chemically clean glass-ware and the highest quality of reagents throughout.

Several recent workers in this field, especially those studying end-bulb or bouton degeneration, have used modification 6*a* of Cajal's method in which chloral hydrate in 10 % solution is used as the primary fixative. This method was found less suitable than modification 4 for two reasons: first, considerable cellular shrinkage occurs, seriously distorting the true relationship between nerve cell and the surrounding neuropil and, secondly, only a small percentage of all end-bulbs present react to the silver. The incomplete nature of the staining with the chloral hydrate methods in our hands rendered any attempt at quantitative estimations by that method much less reliable.

OBSERVATIONS

General appearance of sections

In those sections in which the intensity of silver impregnation is optimum the nervous elements of the grey matter are clearly and selectively stained. Nerve cell bodies, their dendritic processes and the proximal portion of axons stain a light reddish yellow. The fine nerve fibres of the neuropil and the end-bulbs stain more vigorously and stand out in sharp contrast against the cell body and the proximal portions of its processes. The end-bulbs of Held-Auerbach are present in great profusion. The constancy of the picture and the vigorous staining of the terminal branches of the axons and their endings in the best formalin-fixed sections suggest an approximation toward complete impregnation of these finer structures. As will be shown later. the completeness,

quantitatively, with which the end-bulbs stain varies from cell to cell even in the same section.

The nucleus and especially the nucleolus are well stained. The minute spherical bodies of which the latter is composed are clearly seen. Neurofibrillae may be more effectively stained by other modifications of Cajal's method.

The chief sensory portion of the dorsal horn and the whole of the ventral horn of grey matter are suitable for detailed study. The substantia gelatinosa Rolandi stains poorly. No end-bulbs can be seen in this area although they are probably present in large numbers. Similarly the finer details of the nucleus dorsalis are not revealed with the same completeness as in the remainder of the dorsal horn.

General relationships between the end-bulbs and the neurone (Text-fig. 1).

The nerve cell body and the more proximal portions of its processes are embedded in a dense meshwork of nerve fibres. The nervous portion of this neuropil is made up of two types of nerve processes: (*a*) dendrites of neighbouring cells in the grey matter of the spinal cord, and (*b*) axons of neurones whose cell bodies are situated in various regions of the central nervous system and in dorsal root ganglia. The fine terminal and collateral branches of these axons are brought out especially clearly in reduced silver nitrate preparations. Each fine nerve fibre is discrete and there is no suggestion of a nervous network.

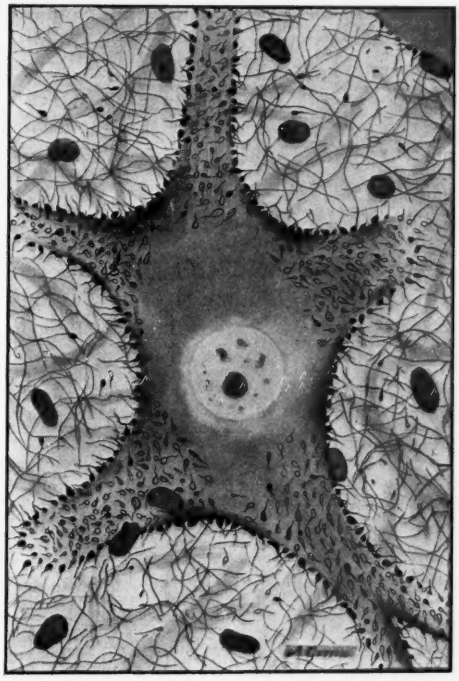
Minute fibres approach the neurone surface at all angles and terminate as small, discrete bulbous expansions on the cell membrane. The end-bulbs are present in large numbers and are evenly distributed over the surface. They are present between the cell membrane and satellite neuroglia cells as well as in the intervals between the latter.

Morphologically there is no apparent difference in the end-bulb-cell membrane relationship between cell body and dendrites. Axon terminations occur with approximately the same frequency and in the same form over the surface of the cell body and the proximal portions of its dendritic processes. Dendrites may frequently be followed 200μ or more from the cell body, and axon branches are still clearly visible terminating as end-bulbs on their surfaces. It is not possible with this technique to determine the nature and form of the synapse at the extreme end of the dendrites. End-bulbs, however, are less frequent in the neuropil than on the cell body and larger dendrites.

Dendrites commonly extend into the white matter of the cord. As observed by Hoff (1932*b*), end-bulbs are visible on these processes even though outside of the limits of the grey matter proper. Synapses, therefore, are not entirely limited to the grey matter of the cord.

It is of theoretical interest to determine the presence or absence of end-bulbs upon the axon. In Cajal preparations it is not always possible to differentiate the axon from the dendrites with certainty. In some cases, however, the axon

may be identified by the abrupt manner in which it arises from the cell body and by its slender, uniform and non-branching shape. In several instances in which such identification was possible, end-bulbs were clearly seen in the region of the axon hillock and the proximal 10–15μ of the axon.



Text-fig. 1. Drawing of a section through a ventral horn cell in the cervical region of the cat's spinal cord. Cajal's reduced silver nitrate method, mod. 4. Mag. app. × 750. Note the large number of end-bulbs on the surface of the cell body and proximal portion of the dendrites. The surfaces of this cell in the plane of the microtome knife lie mainly in adjacent sections

Quantitative estimations

(1) A comparison of the frequency of occurrence of end-bulbs on neurones in various regions of the spinal cord. Particularly good sections were obtained from four cats throughout the whole spinal cord (modification 4 of Cajal's method). These sections were studied intensively from the quantitative standpoint.

Using a binocular microscope with 10× oculars and a 95× oil immersion objective, the sections were searched for cells presenting a surface in the plane of the section. The number of endings situated in the largest rectangular area which the surface might accommodate was counted and the dimensions of the

area were accurately measured with the aid of a filar micrometer eyepiece. In each case the number of endings which would have been accommodated within an area of 100 sq. μ was calculated. In each animal areas on at least ten cells were counted in each of the following regions: (*a*) cells of the ventral horn in the cervical, thoracic and lumbo-sacral regions of the cord; (*b*) cells of the chief portion of the dorsal horn in the cervical and lumbo-sacral regions of the cord. The average figure for all cells studied was seven end-bulbs per 100 sq. μ of cell surface and the average figures for the individual cell groups did not vary beyond the limits of experimental error. These results indicate that the density of end-bulbs on the neurone surface is of the same order in the ventral horn throughout the spinal cord and in the chief portion of the dorsal horn in the cervical and lumbo-sacral regions of the cord. The possibility of minor variations is not excluded.

In the above estimations the cell surface areas were selected at random regardless of the density of end-bulbs on the cell. As a result the counts on single cells varied between three and twenty per 100 sq. μ of cell surface. Silver stained preparations at best are notoriously uneven and it is very probable that the lower counts represent an incomplete staining of the end-bulbs. The average figure of seven endings per 100 sq. μ, therefore, is not representative of the condition in the living spinal cord. It represents, rather, a practical standard for experimental studies when this technique is used.

(2) An estimation of the number of end-bulbs on a motor cell of the ventral horn. In any attempt to estimate the number of end-bulbs on the surface of a cell in the normal, living spinal cord it is necessary to allow for the variability due to the limitations of the staining technique. On the assumption that the lower counts represent cells which were incompletely stained, the sections were searched for ventral horn cells in which the vigorous staining and the large number of end-bulbs present indicated an approximately complete staining of the endings. Counts were made on these cells when a sufficiently large cell surface area lay in the plane of the section. The counts on areas of twenty cells fulfilling these conditions varied between ten and twenty-four per 100 sq. μ with a mean value of sixteen end-bulbs per 100 sq. μ of cell surface. In view of the reputation of the reduced silver nitrate method this figure represents the normal value more closely than that obtained by averaging random counts. Even this higher value is probably too low.

Accurate end-bulb counts on the proximal portion of dendrites are not possible because of the shape of the cell processes. The number per unit area is not greater and appears on general inspection to be somewhat lower than upon the cell body.

Such numerical studies are more informative when expressed in terms of single neurones. The total number of end-bulbs on the surface of a nerve cell body may be roughly estimated. A ventral horn cell fifty μ in diameter has a surface area of approximately 7800 sq. μ. Using the figure sixteen end-bulbs

per 100 sq. μ as representative of the normal average, there would be roughly twelve hundred and fifty end-bulbs on the surface of the cell body alone. It is, of course, impossible to estimate accurately the total number of endings on the cell body and dendrites. On one of the larger neurones of the ventral horn of the cat's cord there would be at least several thousand end-bulbs. These figures emphasize the abundant provision made for synaptic relationships between neurones. The drawing in Text-fig. 1 illustrates the density of end-bulbs on the surface of a well-stained motor cell of the ventral horn.

(3) An estimation of the cell surface area devoted to the synapse. Anticipating the observations below on the size of end-bulbs, the mean size may be taken as approximately 1·5 by 2·0μ. Using the average figure of sixteen endings per 100 sq. μ of cell surface and considering each bulb an ellipse, it may be estimated that approximately 38 % of the surface of the cell body is devoted to the formation of synaptic junctions with other neurones. This corresponds with the general appearance in the most completely stained cells (fig. 1).

Description of the individual endings

The end-bulbs in the grey matter of the cord vary in size from 0·5 by 1·0μ to 4·0 by 5·0μ. The small endings, from 0·5 by 1·0μ to 1·0 by 1·5μ, appear as delicate, well-defined rings or ovals with clear centres. Endings of intermediate size from 1·0 by 1·5μ to 2·0 by 3·0μ are also well-defined rings or ovals. Their centres may be clear and without detail, opaque, or may show a fine fibril passing down the centre of the ending, appearing as a direct continuation of the parent nerve fibre. These suggest that the end-bulb may consist of a continuation of the neurofibrillae of the nerve fibre surrounded by a terminal accumulation of neuroplasm.

The largest endings are much less frequent and vary from 2·0 by 3·0μ to 4·0 by 5·0μ. They may be round, oval or spatulate. The largest end-bulbs are usually opaque and may present a granular or reticulated appearance. Endings of all sizes tend to appear opaque when the cell surface with which they are in apposition is viewed tangentially. The variations in size, shape and appearance of individual end-bulbs are illustrated in Text-fig. 1 and the most common types of end-bulbs appear in the photomicrographs in Pl. I, figs. 1–3. The photomicrographs give no information as to the number of end-bulbs present. Because of the high magnification only one or two endings in a small area appear in the same focal plane.

There is every possible gradation in size from the smallest to the largest end-bulbs. The above description is not to be interpreted as an attempt to subdivide end-bulbs into groups on the basis of variations in size.

The endings described above are included in the term boutons terminaux. In addition, Cajal described similar swellings on the course of nerve fibres and in contact with the surface of a cell body or its dendrites. Such boutons en passant (Cajal), although much less numerous than boutons terminaux, were frequently seen in the sections from the cat's spinal cord.

No differences could be detected in the appearance or distribution of the endings in various segments of the spinal cord. Within the same segment the smaller end-bulbs predominate on the cells of the lateral groups in the ventral horn. Endings of intermediate size predominate on cells of the medial groups of the ventral horn and the chief sensory nucleus of the dorsal horn. The largest end-bulbs occur mainly in company with those of intermediate size. End-bulbs of widely different size and shape may be present within a small area on the same cell.

Observations on the controversial question regarding the ultimate relationship between axon terminations and other neurones are subject to the limitations of the staining technique. The appearance in Cajal preparations is that of intimate contact between the surface membranes of end-bulb and cell body or dendrite. There is no suggestion, with the optical equipment available, of any layer of non-protoplasmic material between the end-bulb and the surface of the cell.

DISCUSSION

Cytologically there is no apparent distinction between the so-called axon-dendritic and axon-somatic types of synapses. In fact, at the base of a dendrite such a terminology is purely arbitrary. These observations bear out the general view that the dendrites are prolongations of cell cytoplasm, differing little cytologically from the cell body proper, but increasing the area of the cell membrane for synaptic and other purposes.

The presence of end-bulbs in the region of the axon hillock is difficult of interpretation. The presence of synapses at the base of the axon is not confined to the mammalian nervous system. Bodian (1937) and others have described end-bulbs and a specialized axon cap in the region of the axon hillock in Mauthner's cell of teleosts.

The numerical studies emphasize the importance of the synapse in the central nervous system. Over one-third of the cell membrane of the neurones especially studied here, exclusive of the axon, may be utilized for the purpose of effecting communications with other neurones. End-bulbs are so numerous that a great many must be derived from a single axon. The functional significance of multiple synapses is not clear. Two possibilities, however, may be suggested. Firstly, a certain minimal synaptic area may be necessary to build up a central excitatory state of sufficient potential to set off a volley of impulses in the next neurone. This area is provided by a large number of small synapses. The intervening cell membrane is then free for the ordinary function of a cell membrane, i.e. the interchange of substances necessary for or incidental to cellular metabolism. Secondly, the physico-chemical change which occurs as a nerve impulse is transmitted from one neurone to another may be in slightly different phase at different end-bulbs. The physiological implications of these suggestions are extensive and of fundamental importance although beyond the scope of an anatomical study.

While only the end-bulb-cell membrane type of synapse has been considered here, it does not follow that other types of inter-neuronal communication do not exist. Of particular interest in this regard is Tiegs' demonstration in new-born rabbits of nerve fibrils in the white matter of the spinal cord passing directly into dendrites of cells in the grey matter of the cord (1926). Tiegs regards the dendrites as a flowing out of grey matter along the incoming nerve fibril. Cajal preparations of adult material do not demonstrate faithfully enough the cytological conditions at the extremities of dendrites. The probability of such a type of interneuronal communication as Tiegs described existing in the mature mammal must, therefore, await further investigation.

It is important to recognize the normal variations which may occur in the size, shape and staining affinity of individual endings. Large end-bulbs of the order of $4{\cdot}5$ by $5{\cdot}0\mu$ frequently occur within a distance of several microns from small endings less than $1{\cdot}0\mu$ in diameter. End-bulbs in such close proximity have been subjected to similar physical and chemical influences during the preparation of the tissue for study. It is concluded, therefore, that the variations described in this paper are based in some measure at least upon variation in the end-bulbs in the living spinal cord and may be used as a standard for evaluating various experimental procedures.

These facts are especially significant in the study of end-bulb or bouton degeneration as a result of sectioning fibre tracts. The occasional occurrence of large endings should not be interpreted as indicative of bouton degeneration as a result of cutting the parent axons since such boutons occur in the normal cord.

The possible causes of the variations in the end-bulbs in apparently normal animals raise important problems which should be subjected to further experimental analysis. Several possibilities are mentioned here as meriting special consideration.

(*a*) The different endings may represent the axon terminals of different types of neurones. The small, delicate endings may represent the axon terminations of Golgi type II association neurones or Golgi type I neurones of the more primitive type with non-medullated or finely medullated axons. The larger endings, on the other hand, may represent the axon terminations of the more highly developed Golgi type I neurones, the cell bodies of which are situated in dorsal root ganglia or higher centres of the encephalon. This suggestion is supported by the observation that smaller endings predominate on cells of the lateral groups in the ventral horn while the intermediate and larger endings predominate on cells of the medial groups in the ventral horn and the chief sensory nucleus of the dorsal horn. Schimert (1938) found vestibulo-spinal fibres to end on the more medial cells.

(*b*) It is not impossible that end-bulbs may undergo some alterations in size, shape or staining affinity with changes in their physiological activity.

(*c*) Several workers, notably Köster (1903), Windle (1927), Allen (1927) and Du Bois & Foley (1937), have reported the presence of chromatolytic cells

in presumably normal cranial and spinal ganglia. On the basis of these observations the occurrence of a number of end-bulbs in the grey matter of the cord in some phase of degeneration is to be expected.

(*d*) At best an artificial picture results from staining small and delicate nerve structures with the reduced silver method. A too literal interpretation of silver sections is, therefore, to be avoided. Unsatisfactory fixation or staining of the cord results in the appearance of many large and granular end-bulbs quite unlike those found in well-fixed and clearly stained preparations.

It has been assumed by many that the neuropil represents the great synaptic region of the central nervous system. There is some experimental evidence to suggest that the neuropil represents as well an area of increased tissue metabolism. Craigie, on the other hand, has recently demonstrated (1938) that in the leopard frog the neuropil may be less vascular than many nuclear areas. It is clear that synapses are by no means confined to the neuropil. If similar conditions exist throughout the central nervous system, axon terminations are present in great abundance upon the cell bodies as well as the dendrites. These facts have an important bearing on the physiological interpretation of vascularity throughout the nervous system.

SUMMARY

1. The morphology of the synapse was studied in Cajal reduced silver nitrate preparations of normal spinal cords from thirty mature cats. Modification 4 of Cajal's technique, involving formalin fixation, was found most generally useful.

2. In cells of the ventral horn and the chief portion of the dorsal horn, axon terminations as end-bulbs are distributed fairly uniformly over the surface of the cell body and the proximal portion of the dendrites. End-bulbs appear to be less numerous on the distal portions of the dendrites.

3. The density of end-bulbs, numerically, on the neurone is essentially the same in cells of the ventral horn and chief portion of the dorsal horn and in the various segments of the spinal cord.

4. The density of end-bulbs on the surface of the cell body of a ventral horn cell is of the order of sixteen end-bulbs per 100 sq. μ by the technique employed. There are several thousand end-bulbs on a single larger motor neurone of the spinal cord in the cat.

5. Approximately 38 % of the cell membrane is devoted to the formation of synaptic junctions with other neurones.

6. Considerable variation exists in the size, shape and staining affinities of individual end-bulbs. The size may vary from less than $1{\cdot}0\mu$ in diameter to $4{\cdot}0$ by $5{\cdot}0\mu$. End-bulbs vary in shape from round to spatulate. The most typical feature of their internal structure is a fine fibril extending the length of the end-bulb appearing as a continuation of the parent nerve fibre.

7. The smaller end-bulbs predominate on cells of the lateral groups of the ventral horn. End-bulbs of intermediate and larger sizes predominate on cells

Fig. 1.

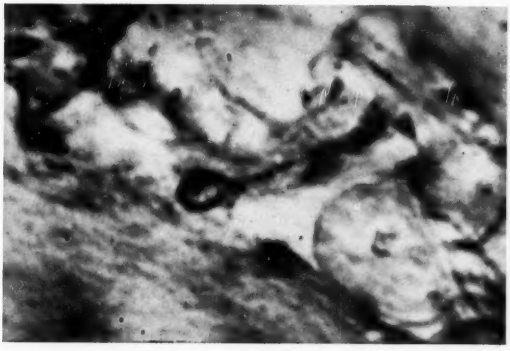


Fig. 2.

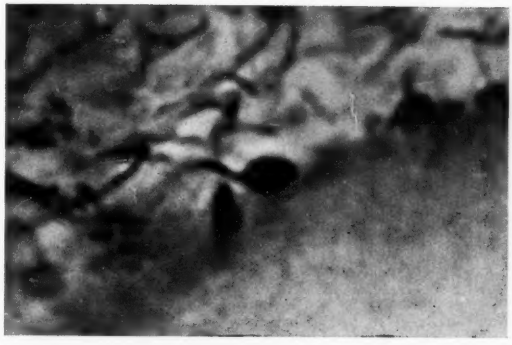


Fig. 3.

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of the medial groups of the ventral horn and the chief portion of the dorsal horn of grey matter.

8. The cytological and physiological significance of the above observations is briefly discussed.

This work was done in the Department of Anatomy under the direction of Prof. H. A. Skinner. The author is indebted to Prof. C. C. Macklin, Prof. F. R. Miller and Prof. A. T. Rasmussen for their interest in these studies.

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EXPLANATION OF PLATE I

Figs. 1–3. Photomicrographs of end-bulbs in the spinal cord of the cat stained by modification 4 of Cajal's reduced silver nitrate technique. Magnification ×2600.

Fig. 1. End-bulbs 1·1 by 2·3μ and 1·7 by 1·9μ on the surface of a ventral horn cell in the cervical region of the spinal cord.

Fig. 2. End-bulb 2·1 by 3·0μ on a dendrite of a ventral horn cell in the lumbo-sacral enlargement. Note the nucleus of a satellite neuroglia cell to right of ending.

Fig. 3. A motor cell in the cervical enlargement has been cut in such a manner that the cell surface is viewed tangentially. The end-bulb centrally placed in the photograph is 2·0 by 3·0μ in size.

AN EXPERIMENTAL INVESTIGATION OF THE MOTOR CORTEX AND ITS CONNEXIONS IN THE PHALANGER, *TRICHOSURUS VULPECULA*

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The Orders of placental mammals show in general a tendency to increase progressively the area and degree of differentiation of the neopallium. This tendency has had its maximum effect in the Order Primates, but is not confined to them. It is a common mammalian heritage which is also shared by marsupials. In many diprotodont marsupials, for example, the neopallium is obviously more extensive, relative to the olfactory cortical areas, than in some of the polyprotodont genera.

Among placental mammals an increase in the area of the neopallium is sometimes accompanied by increased elaboration of the well-known motor cortical area. This may be shown by the way in which the cortical representation of movements of the head and forelimbs, found in all mammals, extends to include the trunk, hindlimbs and tail, and is naturally reflected in the increased bulk and length of the pyramidal tract. The present work was undertaken to determine how far the general increase in neopallial area which has occurred in some marsupials, has been accompanied by changes in the motor cortex.

So far the only published work in which the structure and physiology of the motor area in a marsupial has been investigated in detail by experimental as well as by other methods concerns the polyprotodont, *Didelphys virginiana*. Due mainly to the work of the American school of comparative neurologists, the brain of this animal is known now as thoroughly as that of any primitive mammal. It may conveniently be used as a standard of comparison with the animal chosen for the present study, the phalanger *Trichosurus vulpecula*. In the phalanger the relative extent of the neopallium is much greater than in *Didelphys*, as even a superficial examination of the brain shows. Moreover, as Wood-Jones (1924) has pointed out, the phalanger is the only Australian marsupial that has become adapted successfully to life in competition with such placental mammals as the cat and the fox that the white man has introduced. It is also arboreal in habit. For these reasons it might be expected to have developed a more precise cortical control of movement and behaviour than many of the less successful polyprotodont marsupials.

MATERIAL AND METHODS

The animals used were all adult healthy specimens. A few were smaller than the average, and may not have been quite fully grown; this did not appear to affect the results.

All operations were performed under open ether anaesthesia, and with ordinary aseptic precautions. No unusual difficulties were encountered. The actual exposure of cortex was always as limited as possible, consistent with the purpose of the experiment, and always confined to one hemisphere. Few individual experiments therefore included the full extent of cortex that it is possible to expose in one hemisphere; such limitations in single experiments are largely compensated for by combining the results of several, while the diminished severity of the operation and the shorter time taken increase their reliability. Nearly all the animals were allowed to survive for varying periods, and post-operative complications were negligible.

Stimulation of the cortex was carried out by the unipolar method with alternating current at 50 cycles and a voltage variable up to a limit of 16 V. The apparatus was that described by Myers (1936) and was used successfully in similar experiments on the *Echidna* (Goldby, 1939) and on the cat. The threshold was measured in volts, and with one exception varied between 1·0 and 1·8. In the great majority of experiments it was very constant at 1·1 V. On a few occasions a milliammeter was included in the circuit between the electrodes and showed that at the threshold voltages between 0·1 and 0·3 mA. passed through the animal. These threshold values are of the same order of magnitude as those obtained for the motor cortex of the cat and of the *Echidna*. Care was taken to keep the cortex moist and warm with Ringer's solution between the actual stimulations. The depth of anaesthesia was inevitably variable; it was generally light but sufficient to produce moderate relaxation and to abolish spontaneous movements.

Eleven stimulation experiments were performed, in eight of which the whole excitable area was exposed. There were two further experiments in which only the posterior half of the hemisphere was exposed.

The degeneration experiments fall into two categories: those in which secondary degeneration was studied by the Marchi method after a survival period of 3 weeks, and others in which the nervous system was stained by the Davenport silver impregnation method (1930) after survival periods of 12 weeks or more. The latter experiments were useful in checking the results obtained by the Marchi method. Similar techniques were used by Swank (1936) in experiments on the rabbit.

The cortical lesions were usually made with the diathermy, but in a few cases by scraping away the superficial layer of cortex with a knife. They were all deep, involving more or less of the subcortical white matter, and therefore interrupting projection and other fibres from a wider cortical area than that destroyed. The extent and position of this wider area could be estimated with fair accuracy by an examination of the serial sections into which the specimens were cut. The corpus striatum was undamaged in all the specimens reported.

Six specimens were prepared by the Marchi technique. In five of these cortical lesions had been made, in the sixth, P. XIX, a hemi-section of the spinal cord in the 9th thoracic segment. Only three of the specimens with

cortical lesions are reported, P. VIII, XXII and XXIII. In one of these, P. VIII, the series was incomplete in the forebrain and brain stem owing to failure of staining in some of the blocks, but gave particularly good sections in the spinal cord. On account of similar technical faults the two series not reported were incomplete. They were useful in confirming some of the findings in other specimens, but added nothing on their own account. In all specimens serial sections were prepared from at least three segments of the spinal cord (usually the 7th cervical, the 7th or 8th thoracic and the 2nd lumbar), and cut in celloidin at 40μ. In specimens P. XXII and XXIII the whole brains were cut, and gave satisfactory series. All the sections were kept, and as a routine every 8th was mounted. Where necessary more sections were mounted so as to obtain a more closely graded series in certain regions.

No complete Davenport silver series were prepared, but representative levels in the brain stem and spinal cord were cut and examined. The forebrains were cut serially, as a rule in celloidin, and stained with toluidine blue or cresyl violet to serve in a future investigation of the retrograde cell degenerations in the thalamus. These series have been used in this paper only to determine the extent of the lesion. Two specimens are reported, P. II and P. V. Others were available but they provided no additional information that was relevant.

Transverse, sagittal and horizontal series of normal brains were used to determine the histology and lamination pattern of the motor area. Thick (40μ) celloidin sections were found the most useful; they were stained with toluidine blue. No attempt has been made to prepare a comprehensive cortical map, but only to discover how far the motor area, defined by electrical stimulation, corresponded with a histologically differentiated field.

STIMULATION EXPERIMENTS

Electrical stimulation revealed an excitable area on the dorso-lateral surface of the anterior third of the hemisphere. It was broadest next to the midline where it began 3 or 4 mm. from the frontal pole and ended about 10 mm. from the same point, i.e. approximately a third of the distance from the frontal to the occipital pole of the hemisphere. Laterally it fell short of the rhinal fissure by about 3 mm. (Fig. 1). It was difficult to define the boundaries of this area with any great precision. Under the best conditions it is probably impossible to fix an excitable point with an error of less than 1 mm. in any direction; with an area the greatest breadth of which is 6 or 7 mm. an absolute error of 1 mm. becomes a relative error of between 15 and 20 %. The difficulty is increased by the variations in the excitability of any one point that occur during the course of an experiment. Such variations were common, due no doubt to different causes, such as fatigue, facilitation from adjacent regions, cooling of the cortex or to variations in the depth of anaesthesia. The only sulci in the region are too shallow to be recognizable with certainty during the operation, and cannot be used as reliable landmarks.

No similar movements were obtained from any other part of the cortex that

was explored. This included the superior and lateral aspects of the parietal and occipital regions, and the upper part of the temporal region. With a stimulating voltage higher than the threshold for the motor area a convulsive movement consisting of protraction of both shoulders, raising of the head, and rapid clonic movements of both forelimbs could be elicited from almost any part of the cortex. This movement has an obvious epileptiform character and bears no relation to the responses from a motor area as ordinarily defined.

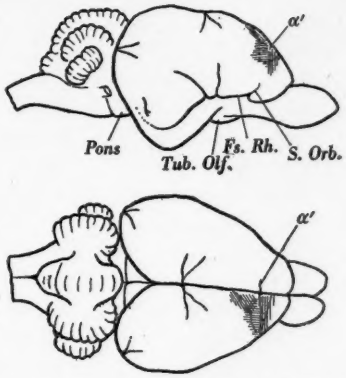

Fig. 1. A dorsal and a lateral view of the brain of *Trichosurus vulpecula*, about natural size. The extent of the motor area, defined histologically, is indicated by the interrupted line; the area defined by stimulation is shown by shading (vertical: movements of head; horizontal: movements of forelimb; oblique: movements of hindlimb).

In the series of eight experiments in which exposure of the motor area was complete movements of the following parts of the body were seen with greater or less frequency: facial muscles, jaws, tongue, neck muscles, forelimb, abdominal muscles and hindlimb. No movements of the tail were seen in any experiment. The movements will be described in the order of the frequency of their appearance.

Forelimb movements were seen in all eight experiments. They were usually complex, involving all the joints of the limb, most commonly a flexion forwards at the shoulder accompanied by flexion of the elbow and digits. Quite often less extensive movements occurred: flexion or extension of the digits, medial or lateral rotation or a slight raising of the whole limb. All these movements were hetero-lateral and were unaccompanied by movement of any other part of the body. A small increase in the strength of stimulation, however, often led to the appearance of homo-lateral in addition to the hetero-lateral movement, or to combined movements of fore- and hindlimb, or of forelimb and head.

Isolated movements of the hetero-lateral hindlimb were seen in six of the eight experiments. They were very similar to the movements of the forelimb; usually complex, e.g. a flexion forwards at the hip combined with flexion or

extension of the digits, but sometimes simpler, such as flexion of the digits alone or slight abduction at the hip. Less variety was observed in the movements of the hindlimb than in the forelimb, but there was not much in either. Increase of the stimulating voltage above the threshold had the same effect as in the forelimb. In the remaining two experiments combined movements of hind- and forelimbs were seen in one, and although the forelimb movement could be obtained alone, the hindlimb movement could not. In the other the only movement obtained at all was of the heterolateral forelimb, and for that the threshold was unusually high, 2·5 V.

Movements in the head and neck were also seen in six of the eight experiments; retraction or protraction of the vibrissae in four, chewing, with or without tongue movements in four, movements of the neck muscles in three and of the eyelids and ear in one each. These movements were all bilateral except for the movement of the ear (contralateral, but seen on one occasion only) and some of the neck movements. All could be obtained isolated from the others, but there was a strong tendency for jaw movements to be combined with movements of the vibrissae. The latter had a particularly extensive representation.

A movement of the trunk musculature was seen in only one experiment. It took the form of a bilateral contraction of the abdominal muscles, as in forced expiration. There seemed no doubt on this one occasion that the movement was caused by cortical stimulation.

It is obvious that the reasons given previously for the difficulty in arriving at any precise definition of the whole motor area in this animal apply with greater force to the attempt to localize movements within the area. Their approximate position is shown in Fig. 1. The points from which movements of the vibrissae were obtained occurred in any part of the head area (vertical shading). Jaw and tongue movements were obtained only from its lateral part. Neck movements resulted from stimulation in the region of overlap between the head and arm areas and the single trunk movement in the similar region between fore- and hindlimb areas. It will be seen that head and forelimb have each quite an extensive representation; the area for the hindlimb, situated posteriorly and near the mid-line, is rather smaller.

The number of experiments is not large enough for the relative frequency of different responses to have much significance. In the later experiments more movements were observed than in the earlier, the result probably of experience and improvement in technique. The variations between different experiments were similar to those noted by Penfield & Boldrey (1937) in a long series of explorations of the human motor cortex. So far as they go, they indicate that movements of the forelimb have greatest cortical representation, followed closely by movements of the hindlimb. Next come movements of the facial musculature (affecting the vibrissae particularly), jaws and neck muscles, all about equally represented as far as ease in eliciting the response is concerned. The representation of movements of vibrissae is spread over the widest area.

Finally the trunk musculature is represented poorly if at all, and there is no representation for the tail.

The most striking characteristic of the movements taken as a whole is their tendency to involve more than one joint. In this way they differ from the more precisely localized movements characteristic of motor cortex stimulation in Primates. Movements of isolated digits, for example, were never seen. Although movements confined to all the digits of either fore- or hindlimb were sometimes seen, it cannot be said that there was any obvious tendency for the movements to occur mainly in the distal segments of the limb. It is possible that these differences from the results obtained by stimulation of the more extensive motor cortex of higher mammals are due to differences of method. It seems more likely that they constitute a genuine characteristic of the motor cortex in relatively primitive mammals, and are partly determined by the small size of the area available for exploration.

HISTOLOGY OF THE MOTOR AREA

The excitable area was found to correspond fairly closely with an area that could be defined histologically. The boundaries of the latter are indicated by the interrupted line in Fig. 1 and other figures. As would be expected, it is smaller than the area defined by stimulation. In it the cortex is about 1·4 mm. in total thickness, and all the six laminae of Brodmann can be recognized. It is mainly characterized by the breadth of the Vth lamina and by the presence in it of typical large pyramidal cells. The IVth lamina is plainly visible, although its boundaries are very ill defined in section. It consists of small polygonal cells, not closely packed, so that in a thin section (10μ) it appears almost as a cell-free layer. The cells are slightly larger and less rounded in appearance than typical granule cells (as seen for example in the IVth lamina in the parietal region). The VIth lamina is broad and fades into the underlying white matter; it contains small polygonal and fusiform cells. The IInd and IIIrd laminae show no very striking characteristics; the cells in them are perhaps larger than in the posterior parts of the cortex and they tend to be of a pyramidal shape. In general the appearance is very similar to that of the same area in the kangaroo, as shown in Brodmann's Fig. 49 (1909); the Vth lamina in the phalanger is rather broader, and the cells of the IInd lamina rather larger.

Anteriorly, covering the dorsal and most of the lateral aspect of the frontal pole the cortex is of a similar type, but all the laminae are thinner except the VIth, and the cells are less closely packed; the Vth lamina lacks large pyramidal cells. It is the change in the character of the Vth lamina which marks the anterior boundary of the motor cortex most clearly.

Posteriorly the boundary is more difficult to define accurately. Taking the characteristics of the Vth lamina as a criterion, one finds a fairly abrupt transition about 10 mm. from the frontal pole close to the mid-line. The lamina becomes much thinner, and although some quite large pyramidal cells are still found in it, they are much fewer in number. The change in character of the

IInd, IIIrd and IVth laminae is more gradual and begins a little anterior to the change in the Vth lamina. It is most marked in the IVth lamina, the cells of which become more numerous and smaller until a well marked granular layer is present. In the IInd and IIIrd laminae the cells lose their pyramidal character and become a little smaller. Laterally the transition in the IVth layer is more abrupt and more clearly defined.

The orbital sulcus of the phalanger (Ziehen's sulcus α, 1897*b*) is not a limiting sulcus to this histologically defined area. Usually it fades away before it reaches the motor area, but when well developed it extends across it to join a second sulcus which runs laterally from the dorso-medial border of the hemisphere. This second sulcus resembles the cruciate sulcus of carnivora in its relation to the motor area. It is Ziehen's sulcus α′. It seems to be always present, but varies greatly in its degree of development.

PHYSIOLOGICAL RESULTS OF CORTICAL INJURY

The following observations were made on animals in which the whole of the motor area and some part of the parietal cortex had been destroyed. In none was there any striatal injury.

A few hours after the operation, when the effects of the anaesthetic had disappeared, there were considerable weakness and clumsiness in both hetero-lateral limbs. They were unable to support the body properly and tended to slip into abnormal attitudes, e.g. extreme lateral rotation in the hind limb, or, in the forelimb, a standing posture in which the wrist and fingers were flexed so that the extensor surface rested upon the ground. A day later there was marked improvement; the animal's gait was nearly normal although the hetero-lateral limbs were used clumsily when it attempted to climb. A week later nearly all these signs had disappeared; control of the flexor movements of the digits of the affected forelimb was still deficient, and the limb was not used in holding food. At the end of 3 weeks no motor disabilities could be detected with certainty.

Rogers (1924) made similar observations on *Didelphys* after destruction of the motor cortex. His findings were very like those recorded here for the phalanger. He used a more detailed series of tests for motor function, which could not, however, be used with the phalanger. None of the animals used in these experiments became at all tame, so that handling was difficult, and one could do little more than observe their behaviour in the cage, or when running freely in the room. If these more detailed tests could have been applied it is possible they would have revealed more persistent motor defects than were actually observed.

DEGENERATION EXPERIMENTS

Before describing the results of these experiments some general characteristics of the lesions must be discussed. Their superficial extent in the five specimens to be reported is shown in Figs. 2, 3, 4, 10, and 11, and their depth

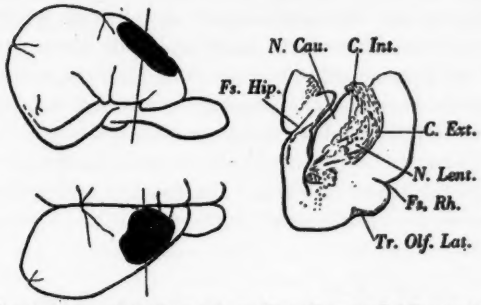

Fig. 2. P. VIII. The drawings of the hemisphere show the superficial extent of the lesion and its relation to the motor area histologically defined. The section (× 2) passes through the middle of the lesion; its level is indicated by the line drawn across the hemisphere.

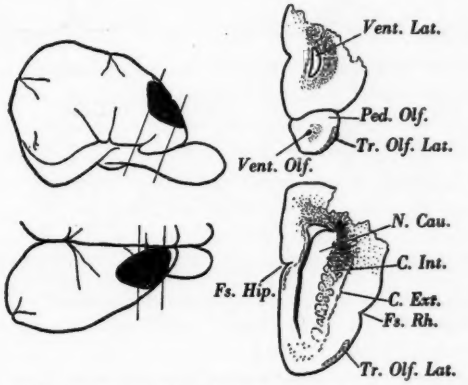

Fig. 3. P. XXII. Similar to Fig. 2, but with two sections through the lesion.

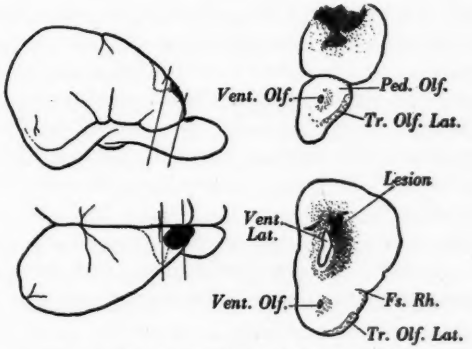

Fig. 4. P. XXIII. Similar to Figs. 2 and 3.

in the accompanying sections. They all extend practically to the ventricle. As a result, all projection fibres connected with cortex lying medial to the lesion must have been divided. In addition, since fibres from the antero-medial parts of the hemisphere pursue an oblique course across the ventricle to reach the internal capsule, some coming from cortex anterior to the lesion will also have been divided. Fibres arising posterior or lateral to the lesion will not be affected. These statements apply also to commissural fibres. The general effect will be as if the lesions reached to the mid-line and included the medial surface of the hemisphere, and reached anteriorly for 1 or 2 mm. in front of the margin shown in the figures.

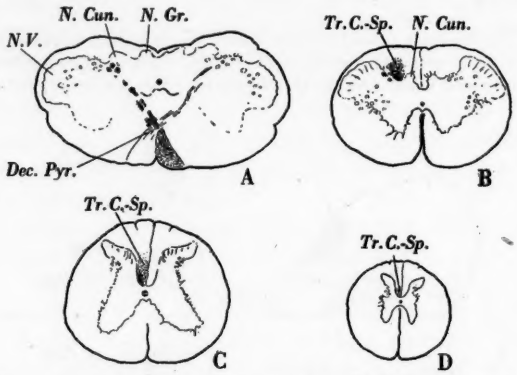

Fig. 5. Four sections showing degeneration in the cortico-spinal tract from the decussation downwards: A, the decussation of the pyramids; B, immediately below the decussation; C, the 8th cervical segment; D, the 8th thoracic segment. All magnified by 4. A is from P. XXII, and B, C and D from P.VIII.

In the nervous system posterior to the pons the only result obtained in any experiment was degeneration of the pyramidal tract. It will be convenient to describe this first.

In specimens P. VIII and P. XXII, all projection fibres from the excitable area were destroyed as well as many from the adjacent regions. In both, the pyramid in the medulla on the same side as the lesion showed definite but not intense Marchi staining. No degenerated fibres could be traced into the reticular formation or into any of the cranial nerve nuclei. At the hind end of the medulla, just anterior to the foramen magnum, the decussation occurs (Fig. 5 A). It is complete, and the fibres run into the posterior column where they form a compact tract lateral to the cuneate nucleus (Fig. 5 B). No evidence was found for the presence of a direct cortico-spinal tract or of cortico-spinal fibres in either anterior or lateral columns. So far the findings are only confirmatory of those of Ziehen (1908) using normal histological material in *Pseudochirus peregrinus*.

As the cuneate nucleus disappears the cortico-spinal fibres sink more deeply

into the posterior columns and come very soon to occupy the position shown in Fig. 5 C, a section through the 8th cervical segment. In the specimen P. VIII degenerated fibres were still clearly present but very much fewer in number in the 8th thoracic segment (Fig. 5 D). In P. XXII degeneration could be traced to the 8th cervical segment, but owing to excessive pseudo-Marchi staining (which affected only the spinal cord and especially the posterior columns), findings below that level were unreliable. As will be seen, the presence of cortico-spinal fibres in the 7th thoracic segment received confirmation in the specimens stained by the silver method. No degenerated fibres were found in the upper lumbar segments in any specimen with a cortical lesion.

The description of degenerations in the nervous system cranial to the pons will be based on the specimens P. XXII and P. XXIII (Figs. 3, 4). The incomplete series available from the specimen P. VIII disclosed nothing relevant to the subject of this communication that could not be seen in P. XXII, except in the spinal cord.

Phalanger XXII (Figs. 3, and 6, 7, 8, and 9, left side)

The extent of the lesion and its relation to the motor area, defined histologically, can be seen in Fig. 3. Degeneration can be followed into the upper part of the internal capsule and into the external capsule. The lower part of the internal capsule, in which run fibres from the frontal pole, is undegenerated.

Two kinds of fibre can be distinguished in the internal capsule; finely myelinated bundles which form the fasciculus aberrans of the anterior commissure (Elliot Smith, 1902), and bundles of coarser projection fibres. The external capsule consists mainly of the fine commissural fibres, but a few small bundles of projection fibres run ventrally in it for a short distance and then turn medially through the lentiform nucleus to join the internal capsule. The commissural fibres form about a third of the internal capsule at this level. Degeneration in them has a fine dust-like character. It can be traced across the mid-line in the upper part of the anterior commissure, and is distributed through the fasciculus aberrans of the opposite side to an area of cortex corresponding approximately to that of its origin. Degenerated commissural fibres in the external capsule behave in the same way, but across in the central and lower parts of the commissure.

The coarser projection fibres pass mainly above but also through and below the anterior commissure (Fig. 6, left side). At this level degeneration still lies mainly in the dorso-lateral part of the capsule, but several practically normal bundles now lie above it. Except in the fasciculus aberrans there is little or no degeneration in the most medial and ventral fibres of the internal capsule.

Behind the anterior commissure the internal capsule begins to enter the subthalamic region (Fig. 7, left side). The degenerated fibres have now been displaced ventro-medially by the entry of a large number of normal fibres from the parietal cortex. At this level fibres connected with the thalamus are beginning to separate from the other constituents of the internal capsule.

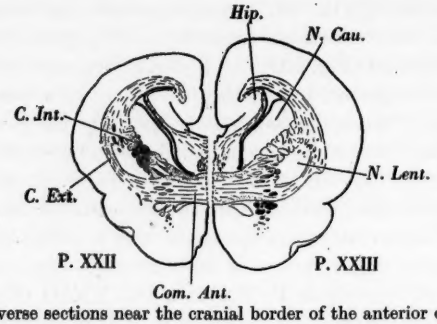

Fig. 6. Transverse sections near the cranial border of the anterior commissure in P. XXII (left) and XXIII (right). Degenerated fibres are stippled (× 2).

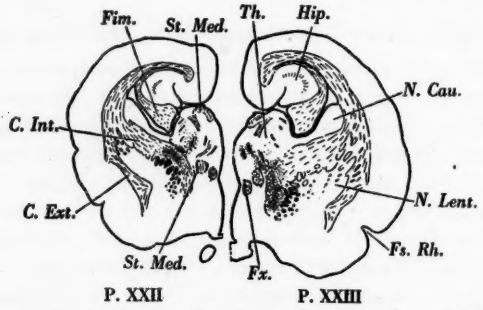

Fig. 7. Sections, arranged as in Fig. 6, to show the distribution of degenerated fibres in P. XXII and XXIII. The sections pass through the anterior third of the thalamus (× 2).

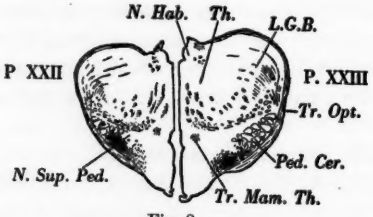

Fig. 8.

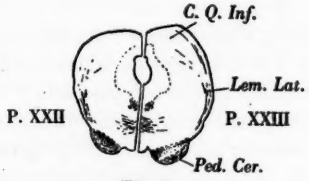

Fig. 9.

Fig. 8. Sections through the caudal end of the diencephalon in P. XXII and XXIII, arranged as in Fig. 6.

Fig. 9. Sections through the mid-brain, just above the pons, in P. XXII and XXIII, arranged as in Fig. 6.

They lie dorso-medially in it and are not divided into clearly defined bundles. Degeneration in these fibres can be traced first into the anterior end of the thalamus, behind this into a position intermediate between the medial and lateral parts of the thalamus, and finally at the hind end of the thalamus into its medial part (Fig. 8, left side). No detailed analysis of the thalamic degenerations is attempted in this paper. A full survey of the normal thalamus is needed for this purpose as well as experimental material in which lesions are more circumscribed.

The other degenerated fibres in the internal capsule are mostly arranged in rather conspicuous bundles. They can be traced through the diencephalon and are shown in Fig. 8 (left side) in the beginning of the cerebral peduncle; here they occupy an intermediate position ventral and lateral to the suprapeduncular nucleus. Some diffuse Marchi staining in the subthalamus above them suggests that a few of these fibres end here. This diffuse staining becomes much more marked in the cranial part of the tegmentum of the mid-brain, where also a few discrete fascicles (degenerated) leave the peduncle to run caudally in the tegmentum, at first ventro-medial to the medial geniculate body. At the upper border of the pons these fascicles turn dorso-medially and are lost in the neighbourhood of the motor nucleus of the trigeminal nerve. As a result of this loss of fibres degeneration in the peduncle at the upper border of the pons is less intense than it was at the beginning of the mid-brain.

During its passage through the mid-brain some rearrangement takes place among the fibres that remain in the peduncle. The lateral undegenerated bundles run medially, ventral to the others (Fig. 9), and can be followed into the transverse fibre system of the pons. They are presumably the temporopontine tracts, but they may well arise from the parietal cortex also. In the remaining part of the peduncle the degenerated fibres become diffusely scattered and can be followed through the pons into the medullary pyramid whence their further course has already been described. No degenerated fibres appeared to end in the pons.

Phalanger XXIII (Figs. 4, and 6, 7, 8 and 9, right side)

In this specimen fibres connected with the dorsal and medial side of the frontal pole have been damaged. The lesion also encroaches on the anterior part of the excitable area, from which movements in the head and neck were obtained. There was slight damage to the olfactory bulb which led to a little degeneration in the lateral olfactory tract.

Degeneration in the internal capsule was practically confined to its ventral and medial part. So far as the commissural fibres were concerned there was no great difference from P. XXII, except that more degeneration was seen in the ventral part of the commissure. At the level of the anterior commissure it is mostly the sub- and intracommissural bundles of the internal capsule that are degenerated (Fig. 6, right side), but a few fibres above the commissure are also affected. In a section through the anterior end of the thalamus degenera-

tion is present in both the cortico-thalamic and other projection fibres, and is found in the ventro-medial part of the internal capsule (Fig. 7, right side). Degenerated thalamic connexions can be traced almost exclusively into the dorso-medial part of the thalamus throughout its whole length. The other degenerated projection fibres take up a position in the most medial part of the cerebral peduncle (they are seen in Fig. 8, right side, ventro-medial to the supra-peduncular nucleus) and rather more of them appear to end in the subthalamus and fewer in the tegmentum than in P. XXII. No degenerated fascicles were seen passing to the neighbourhood of the trigeminal nuclei. As a result of the rearrangement that takes place in the mid-brain degenerated fibres come to occupy a position next to the tegmentum just above the pons. There is some concentration medially and laterally, with fewer in an intermediate situation (Fig. 9, right side). Certainly most and perhaps all these degenerated fibres can be followed through the pons and are found evenly distributed through the one medullary pyramid. They are of course very much fewer than those found in this position in P. XXII. Some are still found in the pyramidal decussation, but they can no longer be identified in the 7th cervical segment of the spinal cord.

Phalanger XIX

In this animal a hemi-section of the spinal cord was performed in the 9th thoracic segment. Serial sections through the lesion showed afterwards that both posterior columns had been damaged, one only slightly and in its superficial part. The lateral and anterior columns were divided only on the one side.

Immediately above the lesion in the 8th thoracic segment degeneration was complete in the posterior column on the side of the hemi-section with the exception of a small oval area medial to the root of the posterior horn. The position corresponded with that of the degenerated fibres seen in the 8th thoracic segment in P. VIII. Higher in the spinal cord it was no longer conspicuous, being surrounded by normal ascending fibres which had been added from nerve roots higher than the lesion.

In the segment immediately below the lesion (the 10th thoracic) degeneration was also present in the posterior columns mostly adjacent to the posterior horn of grey matter. Five segments lower (the 2nd lumbar) it had disappeared except in the deepest part of the posterior column. Here it was unilateral (on the same side as the lesion) if some much less marked stippling on the opposite side may be taken to be a pseudo-Marchi reaction.

It is clear that the results of this experiment are not conclusive. They are consistent with the supposition that descending fibres are present in the deepest part of the posterior columns and that they extend as low as the 2nd lumbar segment. The degeneration in the posterior columns below the lesion is probably mainly the result of damage to short descending branches of the posterior column fibres. That it persists in the deepest part of the posterior column five segments below the lesion, and is absent from the same situation

above the lesion, is in favour of the interpretation that long descending fibres also occupy this position.

Phalanger II (Fig. 10)

In this specimen 12 weeks were allowed for degeneration and selected levels in the brain stem and spinal cord cut and stained by the Davenport silver method.

The lesion was extensive, and situated postero-lateral to the motor area. It was deep, and must have divided all the projection fibres from at least the posterior three-quarters of that area as well, of course, as many others.

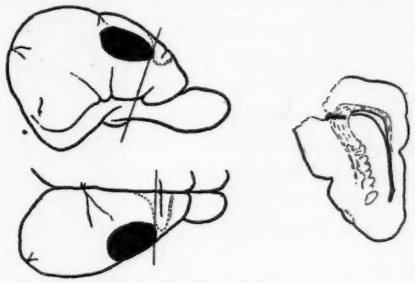

Fig. 10. P. II, showing the superficial extent of the lesion and a section near its anterior border.

Sections showed that on the normal side the pyramidal tracts throughout their course consisted very largely of non-myelinated fibres. This no doubt accounts for the lack of intensity in the Marchi staining in other specimens. It is similar to the condition in the rat (Ranson, 1913), where, however, myelination is probably less than in the phalanger.

On the side of the lesion the loss of fibres in the medullary pyramid was very marked although not complete. The difference from the normal side was very obvious in the decussation and in the hetero-lateral posterior column of the cervical part of the spinal cord. It was still quite clear in the mid-thoracic region, but could not be detected in the upper lumbar segments. In this respect the specimen confirms the Marchi results but does not extend them.

Phalanger P.V. (Fig. 11)

This specimen was prepared in the same way as P. II. It is included mainly as a control.

The lesion was again deep and extensive. It lay in the lateral parietal region and anteriorly fell about 4 mm. short of the level reached by the lesion in P. II.

In the medulla, where the pyramids are compact and easy to compare with each other, there did appear to be a slight but not very consistent difference between them, that on the normal side being a very little larger. In the

peduncles in the mid-brain the difference between the two sides was rather more marked, and seemed to affect the lateral more than the medial fibres. No significant difference could be observed in the cervical, thoracic or upper lumbar segments of the spinal cord.

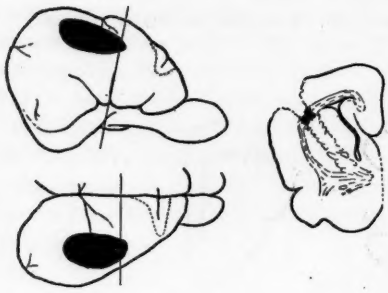

Fig. 11.

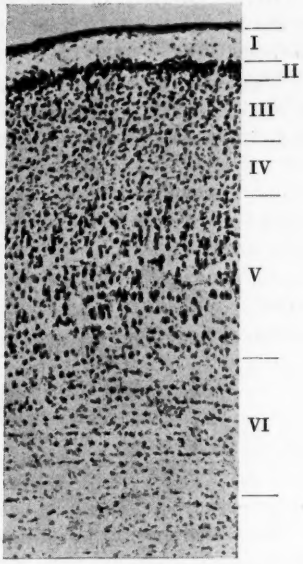

Fig. 12.

Fig. 11. P. V, showing the superficial extent of the lesion and a section near its anterior border.
Fig. 12. A photograph of a section of the cortex about 2 mm. from the mid-line and just behind the sulcus α'. The section is 40μ thick, and the stain toluidine blue; $\times 45$.

On the basis of this specimen it may at least be said that no considerable number of pyramidal tract fibres arise from any part of the cortex that could have been affected by the lesion, i.e. the greater part of the parietal region.

DISCUSSION AND CONCLUSIONS

In broad outline the conclusions that can be drawn from this series of observations are clear and definite. There is in the anterior third of the hemisphere of the phalanger a cortical area that responds to electrical stimulation in the same way as the motor area of eutherian mammals; movements of the hindlimb are represented in this area as well as movements of forelimb, head and neck, and possibly trunk. The frontal pole of the hemisphere is inexcitable. The area defined by stimulation corresponds roughly with a cytoarchitectonic area which possesses many of the characteristics of Brodmann's Area gigantopyramidalis, but shows the full number of six laminae more clearly than is usual in this region. The cytoarchitectonic area is considerably smaller than

the excitable area; it is doubtful if the difference is greater than can be accounted for by the spread of the stimulating current beyond the actual point to which the electrode is applied.

Degeneration experiments have shown that cortico-bulbar and cortico-spinal fibres arise from the cortex in this anterior third of the hemisphere, but not from the inexcitable part of the frontal pole (with the possible exception of a few cortico-bulbar fibres). Because the lesions were large it is not possible to make a more precise statement, although it is very probable that they arise predominantly from the excitable area. In the internal capsule and at the beginning of the peduncle they occupy a position similar to that in other mammals. Above the pons, temporo- (and perhaps parieto-) pontine fibres from the lateral part of the peduncle run medially on its superficial aspect to enter the pons, while the degenerated cortico-spinal and cortico-bulbar fibres become fairly evenly spread through the rest of the peduncle. They pass through the pons and pyramids of the medulla, decussate in the usual situation into the opposite posterior column of the spinal cord, and can be followed caudally as far as the mid-thoracic region. Many, probably most, of these fibres are non-myelinated. Some cortico-bulbar fibres leave the peduncle in the mid-brain and run caudally in the tegmentum, probably to connect with the motor nuclei of cranial nerves.

Some information about the cortico-pontine connexions can be gained from this material. At least the lateral third of the peduncle at the beginning of the mid-brain is occupied by fibres which remain undegenerated after extensive destruction of the frontal cortex and some part of the anterior parietal region. The destination of these fibres in the pons has already been described. A lesion confined mainly to the frontal pole leads to degeneration in the most medial part of the peduncle at its beginning, but few if any of these fibres can be traced to a termination in the pons. Certainly most of the degenerated fibres that can still be seen in the peduncle at the upper border of the pons in P. XXIII are still present in the pyramid below the pons. As far as myelinated fibres are concerned, therefore, the main connexions of the dorsal and medial parts of the frontal pole are with the thalamus, subthalamus and tegmentum. Fronto-pontine fibres are presumably non-myelinated, or lose their myelin sheaths some distance above the pons. None of the material described here gives any evidence of their presence. They are probably few in number, the predominant cortico-pontine connexions coming from the posterior two-thirds of the cerebral cortex.

Some cortico-thalamic connexions have been disclosed, but for reasons given they have not been analysed in detail. Two features may be pointed out however. Firstly, degenerated fibres entering the anterior nuclei were more abundant in P. XXII than in P. XXIII. In the former, more fibres from the medial surfaces of the hemisphere, i.e. the cingulate area, have been divided than in P. XXIII, and it is this region of the cortex that has been shown in some placental mammals to have predominant connexions with the anterior

thalamic nuclei (le Gros Clark, 1932, 1933). Secondly, it was seen in P. XXIII, where the lesion was mainly in the frontal pole anterior to the motor area, that degeneration in the thalamus ran chiefly into its dorso-medial part. In P. XXII, where much of the pre-motor cortex was spared, it ran further laterally in all but the most caudal part of the thalamus. The dorso-medial course of the degeneration seen in P. XXIII is very similar to that described by le Gros Clark (1932) in a rat with a more limited lesion of the frontal pole. In that case it led to the dorso-medial nucleus of the thalamus. It seems therefore that there are some close similarities between placental and marsupial mammals in their cortico-thalamic connexions, a matter that obviously needs further study in the marsupial.

These conclusions can now be compared with those reached by other workers in the opossum, *Didelphys virginiana*. The excitable area in the phalanger differs from that in the opossum (Gray & Turner, 1924) by the presence of cortical representation for movements of the hindlimbs. In other respects it is closely similar, e.g. in the wide representation for movements of the vibrissae in the anterior part of the area, of jaw and tongue movements laterally and nearer to the rhinal fissure, and of the forelimb in the posterior part of the area. The character of the movements appears to have been much the same. Retraction of the vibrissae and erection of the ear were not obtained in these experiments from the parietal and occipital region as in the opossum. This may be due to the fact that the posterior parts of the cortex were explored in only a few cases, and is not an important discrepancy. The results obtained by Rogers (1924) do not differ except in detail from those of Gray and Turner. In short, the only outstanding difference between the phalanger and the opossum is the cortical representation of movements of the hindlimb in the former but not in the latter. Since the movements in question were obtained in six out of eight experiments and at a threshold no higher than for other parts of the motor area, it does not seem possible to explain them as the result of the diffusion of stimulus to subcortical structures, or as fortuitous movements occurring under light anaesthesia. It is true that some early experiments by C. L. Herrick & Tight (1890) and Ziehen (1897*a*) suggest the possibility of hindlimb representation in *Didelphys*. They have been adequately discussed by Gray & Turner, whose results appear to be more reliable.

The comparison of the cytoarchitectonic area in the phalanger with that in the opossum (Gray, 1924) is rather more difficult. It seems probable that the area mapped in the phalanger corresponds with Gray's area pre- and postorbitalis, although there is no clear-cut differentiation of a posterior granular from an anterior agranular part. In general, the lamination is far more distinct in the phalanger. The IVth layer, although not typically granular, is easily recognizable throughout, and the Vth layer is much thicker. It is clear also that the cortex covering the frontal pole in the phalanger is far more highly differentiated than in the opossum (Gray's area frontalis). Abbie has previously noted the presence of a IVth lamina in the motor cortex of monotremes

(1938), and also in the phalanger and other Australian marsupials in work at present in course of publication. Its significance will receive discussion there and need be considered no further in this paper.

In comparing the pyramidal tract of the phalanger with that of *Didelphys* a clear-cut difference is at once found. Turner (1924), using the Marchi method and purely cortical lesions, was unable to trace these tracts below the decussation at the beginning of the spinal cord. He states further that sections stained by the pyridine silver and Cajal methods failed to reveal an unmyelinated cortico-spinal tract below this level. In the phalanger both myelinated and non-myelinated cortico-spinal fibres can be demonstrated easily in the lower cervical segments and in smaller numbers in the mid-thoracic segments. In the position of the cortico-spinal tracts in the brain-stem, and in the level and form of the decussation, the phalanger and the opossum do not differ significantly.

It seems probable that cortico-spinal fibres in the phalanger do not extend far below this mid-thoracic region, certainly not in numbers sufficient to be demonstrated by the methods used. At the same time the specimen P. XIX, with a hemi-section of the spinal cord in the 10th thoracic segment, provided some evidence for the presence of long descending fibres in the posterior columns as far down as the 2nd lumbar segment. If this should prove correct, they may not be of cortical origin. Swank (1936), in the rabbit, showed that some of the fibres in the pyramids arose from the corpus striatum, and that these fibres of striatal origin descended further than the cortico-spinal tracts. Similar fibres may be present in the phalanger. It was noticed that in P. II a fair number of normal fibres still remained in the degenerated pyramid, but more experiments are needed to determine their origin.

Loo (1931) has given a very detailed account of the forebrain of the opossum based on normal material. In most respects the experimental findings recorded here are in close agreement with his description, e.g. in the position occupied by fibres from the frontal pole and from the motor area in the internal capsule and the cerebral peduncle. The rather diffuse degeneration seen entering the subthalamus and the tegmentum correspond with his subthalamic radiation and the radiation of Forel respectively. No further light is thrown on these radiations by the experiments, except to show that they arise in part at least from the frontal third of the hemisphere, and that from the frontal pole fibres enter the subthalamus in greater numbers than the tegmentum. There are two main differences between Loo's findings and the experimental results in the phalanger. The only connexion described by Loo for the anterior thalamic nuclei is the "anterior thalamic radiation" which he traces to the lateral part of the frontal cortex. The degeneration experiments suggest that the main connexions of this nuclear group in the phalanger run to more medial parts of the cortex posterior to the frontal pole. As was said earlier, this point needs further investigation.

The remaining difference between Loo's findings and the experimental

results lies in the interpretation of the most medial fibres in the cerebral peduncle. These certainly come from the frontal cortex (his "medial frontal connexion"), but I have been unable to trace them to a termination in the pons, while he states that they terminate in the medial pontine nucleus. It is doubtful if the Marchi technique is capable of deciding this point with certainty. The fronto-pontine connexions may all be non-myelinated. In spite of this uncertainty, Loo's findings, as well as my experimental results, have some bearing on Abbie's account of the general significance of cortico-pontine connexions (1934). In both the opossum and the phalanger at least the lateral third of the peduncle is occupied by temporo- and possibly parieto-pontine fibres, and even if a large non-myelinated fronto-pontine tract is present it certainly has not displaced the cortico-spinal tracts to the extreme lateral border of the peduncle. It is therefore impossible to conclude, at least in these two animals, that the pre-trigeminal position of the pons has anything to do with an exclusive or even a predominant development of the fronto-pontine tract.

Little is added by these experiments to our knowledge of the commissural connexions in the marsupial brain. Fibres which cross in the anterior commissure are shown to be present in the anterior part of the internal capsule as well as in the external capsule, a well-known characteristic in the brain of diprotodont marsupials (Elliot Smith, 1902; Abbie, 1937). They arise in the frontal pole which they connect with the corresponding region on the opposite side. It may be added that in no specimen was any degeneration seen in the hippocampal commissure that could not be interpreted as artefact, or as the result of slight injury to the alveus fibres across the ventricular cavity. This of course does not prove that neopallial fibres do not enter the hippocampal commissure. The lesions do not cover the whole cortex even in the anterior third of the hemisphere. They show however that, if they are present at all in this region, they can extend no further than the dorso-medial margin of the hemisphere, and no confirmation is provided for Johnston's findings in *Didelphys* (1913).

Only a few reports of similar work in other marsupials are available for further comparison. C. & O. Vogt (1907) defined the motor cortex in another species of opossum, *Didelphys marsupialis*. Their paper is not available in Australia, but from the summary given by Gray & Turner (1924) the results seem very similar to these in the phalanger. Movements of the vibrissae have a less extensive representation. Movements of the hindlimb are represented in a similar position relative to the other parts of the motor cortex but rather far back in the hemisphere, and are accompanied by a representation for tail movements. Flashman (1908) produced a similar map of the motor cortex in *Dasyurus viverrinus*. It appears to occupy a far more restricted area in this animal, being practically confined to the posterior lip of the orbital sulcus. Movements of the head were represented below and not in front of those of the limbs; they were frequently complex, e.g. snarling, or movements of attack. He found a definite representation for hindlimb movements, above and behind that for the forelimb. It is obvious that differences in detail between the results

of different workers, not using identical techniques, are of little significance. The presence or absence, however, of representation for movements of an important part of the body cannot be considered a detail. In both these investigations representation for movements of the hindlimb has been found, in contrast to its absence in *Didelphys virginiana*. From what one can discover about the technique and care employed it seems unlikely that the finding is due to diffusion of the stimulus to subcortical structures or to fortuitous movements, but in the absence of confirmation no more can be said.

It may be concluded that the increase in the extent of the neopallium relative to other parts of the brain that has occurred in *Trichosurus vulpecula*, as compared with *Didelphys virginiana*, has been accompanied by an increased representation for movements of different parts of the body in the motor area. This is shown by the development of an area from which movements of the hindlimb can be elicited and by the greater length of the pyramidal tract. The work of C. & O. Vogt (1907) and of Flashman (1908), although at present lacking confirmation, suggests that a similar development has occurred in the motor cortex of two polyprotodont marsupials, *Didelphys marsupialis* and *Dasyurus viverrinus*. Judging from published figures, there appears to be some increase in the total neopallial area in both these animals as compared with *Didelphys virginiana*, but probably very little. Such as it is, therefore, evidence from the marsupials suggests that the motor area of the cortex may increase so as to include representation for movements of the hindlimb, without much corresponding increase in the total neopallial area. In other words, there does not appear to be any close correlation between the degree to which the motor area and pyramidal tract are developed, and the area of the neopallium as a whole.

Among placental mammals the degree of development of the pyramidal motor system shows an even more remarkable independence of the total neopallial area. In the rat the cortico-spinal tract can be traced as far as the sacral segments of the spinal cord, and it lies in the posterior column (King, 1910; Ranson, 1913). In the rabbit it ends in the upper cervical segments (Swank, 1936). Again, in the sheep, with a highly convoluted and extensive neopallium, it cannot be traced beyond the 1st cervical segment (King, 1911), while in a monotreme, the *Echidna*, it can be followed in the lateral column at least as far as the upper lumbar region (Goldby, 1939). Phylogenetically the pyramidal motor system is extremely unstable and the factors that have influenced its development are at present obscure.

ACKNOWLEDGEMENTS

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SUMMARY

An electrically excitable area has been found in the cortex of the anterior third of the hemisphere in the phalanger, *Trichosurus vulpecula*. It falls short of the frontal pole, which is inexcitable, by about 3 mm. In it, movements of the head and neck, forelimb, possibly trunk, and certainly hindlimb, are represented. The threshold for effective stimulation is similar to that for the motor cortex of other mammals.

The area defined by stimulation corresponds with a cytoarchitectonic area, but is rather larger. The latter shows most of the characteristics of Brodmann's Area 4, but the IVth lamina, usually absent in this area, is present although not typically granular.

Destruction of the motor area leads only to transient motor disabilities in the hetero-lateral limbs.

Marchi experiments have shown that a cortico-spinal tract arises within the anterior third of the hemisphere, probably from the motor area, and after a complete decussation at the hind end of the medulla, extends in the posterior column at least as far as the mid-thoracic segments of the spinal cord. They suggest that cortico-thalamic fibres from this part of the hemisphere are similar to those in placental mammals, but that fronto-pontine fibres are scanty or non-myelinated.

The Marchi results, so far as they concern the cortico-spinal tracts, have been checked by experiments in which 12 weeks were allowed for degeneration, after which parts of the nervous system were stained by the Davenport silver method. These experiments showed that many cortico-spinal fibres are non-myelinated.

The results have been compared with those from other marsupials, particularly *Didelphys virginiana*. The chief difference from the latter lies in the greater length of the cortico-spinal tract and in the representation of hindlimb movements in the cortex.

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ABBREVIATIONS USED IN THE FIGURES

C.Ext.	External capsule	*N.Lent.*	Lentiform nucleus
C.Int.	Internal capsule	*N.Sup.Ped.*	Supra-peduncular nucleus
C.Q.Inf.	Inferior corpus quadrigeminum	*N.V.*	Spinal nucleus of the trigeminal nerve
Com.Ant.	Anterior commissure		
Dec.Pyr.	Decussation of pyramids	*Ped.Cer.*	Cerebral peduncle
Fim.	Fimbria	*Ped.Olf.*	Olfactory peduncle
Fs.Hip.	Hippocampal fissure	*S.Orb.*	Orbital sulcus
Fs.Rh.	Rhinal fissure	*St.Med.*	Stria medullaris
Fx.	Fornix	*Th.*	Thalamus
Hip.	Hippocampus	*Tr.C.-Sp.*	Cortico-spinal tract
L.G.B.	Lateral geniculate body	*Tr.Mam.Th.*	Mammillo-thalamic tract
Lem.Lat.	Lateral lemniscus	*Tr.Olf.Lat.*	Lateral olfactory tract
N.Cau.	Caudate nucleus	*Tub.Olf.*	Olfactory tubercle
N.Cun.	Cuneate nucleus	*Tr.Opt.*	Optic tract
N.Gr.	Gracile nucleus	*Vent.Lat.*	Lateral ventricle
N.Hab.	Habenular nucleus	*Vent.Olf.*	Olfactory ventricle

EXPERIMENTS ON THE DEVELOPMENT OF THE AMPHIBIAN MESONEPHROS

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In *Amblystoma* the normal development of the nephric system has been described by Hall (1904) and Field (1891). The pronephric rudiment appears soon after the closure of the neural folds and consists of a collection of cells derived from the mesoderm of the third to seventh body segments, the rudiment lying at the ventro-lateral border of the somites. By vital staining methods (O'Connor, 1938) it has been shown that the portion of the pronephric rudiment derived from the third and fourth segments forms the pronephros. The more caudal portion of the rudiment (derived from segments five to seven) undergoes an independent growth in a caudal direction and unites with the cloaca, thus forming the pronephric duct. At first both the original rudiment and its caudal extension lie at the ventro-lateral border of the somites immediately beneath the ectoderm and so give rise to a swelling visible from the exterior.

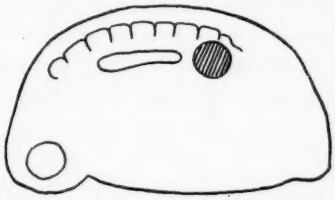
Hall's investigations on the development of the mesonephros show that it appears some little time after the pronephric duct has united with the cloaca, and its differentiation begins with the demarcation of clumps of cells from the surrounding mesoderm in the ninth to eighteenth body segments. These clumps of cells lie on the medial side of the pronephric duct and are arranged segmentally in relation to the posterior quarter of each myotome. In subsequent development the pronephric duct and these clumps of mesonephric cells undergo an inward migration and come to lie close to the mid-line immediately ventral to the notochord. During this migration, however, the close contact between the mesonephric cells and the pronephric duct is maintained. The mesonephric units differentiate into simple tubules which communicate with the pronephric duct which then becomes the draining duct of both the pronephros and mesonephros. The mesonephric tubules become more and more coiled, eventually giving rise to the complicated tubular structure of the mesonephros. As differentiation proceeds the original segmental arrangement of the mesonephric units is lost. Although the mesonephric units appear simultaneously, differentiation proceeds in a cephalo-caudal direction; thus in *Amblystoma*, after about 30 days' development, it is possible to illustrate the main stages in the development of the mesonephros. In a section through the caudal end of the mesonephros differentiation of the mesonephric units into tubules has not yet begun and the mesonephros is represented by clumps of cells in contact with the medial aspect of the pronephric duct (Pl. I, fig. 1). In the middle of the mesonephric region tubules have begun to form, and this is about the stage at which the mesonephric tubules communicate with the pronephric

duct (Pl. I, fig. 2). At the anterior end of the mesonephros, tubule formation is well advanced (Pl. I, fig. 3).

Thus it is seen that throughout development there is a close functional and topographical relationship between the mesonephros and pronephric duct; consequently the question arises as to whether there is any influence exerted by one structure on the other during development. In order to investigate this question, experiments were devised in which the mesonephros was allowed to undergo its development in the absence of the pronephric duct on the same side. In this way it was possible to test the developmental potencies of the mesonephros in the absence of the pronephric duct and thus gain information in regard to the influence of the pronephric duct in the development of the mesonephros. This type of experiment consisted in preventing the formation of the pronephric duct by obstructing the caudal extension of the pronephric rudiment that gives rise to it.

MATERIAL AND METHODS

The majority of experiments were performed on a species of *Amblystoma* which is kept in the Zoological Department of University College, London. In the past this species has been referred to as *A. tigrinum*, but recently doubts have arisen as to the correctness of this name; in this communication, therefore, it will be referred to as *Amblystoma* without defining the species. The caudal growth of the pronephric rudiment was prevented by a suitably placed transplant at the stage when the pronephric rudiment first became visible as an external swelling. The transplant was then placed at the caudal limit of the swelling and caudal extension thus prevented. Since the transplanted tissue merely acted as a mechanical obstruction, the type of tissue transplanted was not of importance, and neural plate, limb bud, eye vesicle, pronephros and cloaca were used for this purpose. In *Amblystoma* the appropriate stage for the experiment is stage 22 (Harrison),[1] in which the pronephric rudiment gives rise to a swelling immediately ventral to the third to seventh somites. The transplant was placed immediately ventral to the seventh and eighth somites, and thus did not disturb the mesonephros which develops in the ninth to eighteenth segments (Text-fig. 1).



Text-fig. 1. *Amblystoma*, stage 22. Position of transplant to obstruct caudal growth of pronephric rudiment.

The technique of transplantation is well known and the method used was essentially that of Spemann (1920). However, certain modifications have proved useful. A preliminary sterilization of instruments and vessels limited operative mortality. No attempt was made to sterilize the water used, which

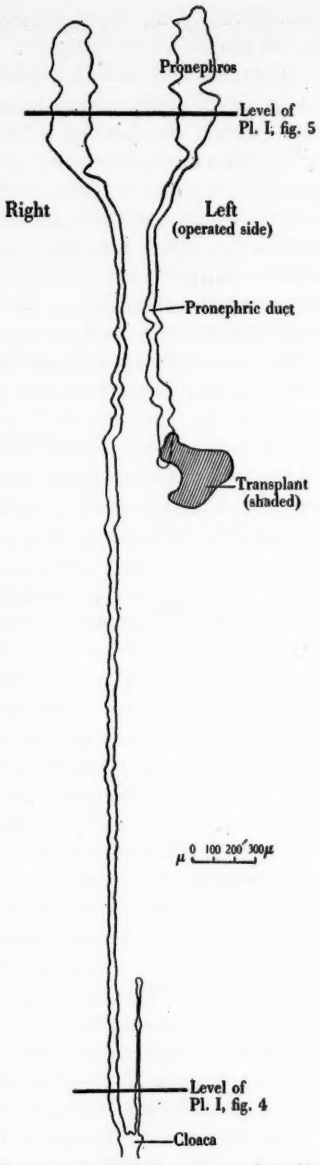
[1] Harrison's numbered stages refer to the development of *A. punctatum*, but the development of the species of *Amblystoma* used is sufficiently similar to warrant their application here.

was taken direct from the tap, but instruments and vessels were kept in 90% alcohol and washed in running tap water immediately before use. This represents a modification of sterilization by formalin vapour as used by Woerderman (1930). In some cases the glass needles of Spemann's method were replaced by fine knives as described by Brandt (1937).

RESULTS OF OBSTRUCTING THE CAUDAL GROWTH OF THE PRONEPHRIC RUDIMENT

In Text-fig. 2 are shown diagrammatically some of the results of blocking the caudal growth of the pronephric rudiment. The experiment consisted in transplanting the cloaca and surrounding tissues from a donor at stage 22 to a position immediately ventral to somites 7–8 on the left side of an embryo at the same stage of development. The embryo was killed eighteen days later and examined by serial sections. The accompanying diagram was made by drawing the sections by means of a projection microscope, measuring the width of the structures as well as their distance from the mid-line and plotting these measurements to scale on squared paper. On the operated side the transplant is shown shaded, and it is seen that the pronephric duct ceases at the transplant and is not found immediately caudal to it. However, at the caudal end of the embryo there is a small tag of duct-like structure attached to the cloaca. In a section through this tag (Pl. I, fig. 4) its structure is demonstrated and both in position and histological appearance it resembles the pronephric duct. Although this was not a constant finding, it does suggest the possibility of a contribution from the cloaca to the hindermost portion of the pronephric duct.

The pronephros in this experiment, and in all other similar experiments, shows no modification in development although the natural outlet for its secretion is blocked. A section taken through the pronephros



Text-fig. 2. Nephric system of *Amblystoma*. 18 days previously caudal growth of pronephric rudiment obstructed by a transplant. Transplant shown shaded. Levels of Pl. I, fig. 4, and Pl. I, fig. 5, indicated.

(Pl. I, fig. 5) shows neither atrophy nor dilatation of tubules. The obstructed pronephric duct, on the other hand, undergoes a variety of modifications when the caudal growth of the pronephric rudiment is prevented, and these modifications are illustrated by sections taken from several different experiments. Immediately cranial to the obstruction the pronephric duct may undergo considerable hypertrophy and dilatation (Pl. II, fig. 1), while in (Pl. II, fig. 2) is shown a communication with an artificially produced diverticulum of the gut. In addition there have been observed communications with the exterior (Pl. II, fig. 3) and with a cloaca transplanted into the flank of the embryo (Pl. II, fig. 4).

In all such experiments the development of the host cloaca is unaffected by the absence of the pronephric duct on one side and Pl. II, fig. 5, illustrates the symmetry of the cloaca in these circumstances. Particular attention is drawn to the diverticula present on either side. The pronephric duct normally joins these, but even in the absence of the duct the diverticulum forms normally. Its formation is apparently independent of the presence or absence of the pronephric duct.

THE DEVELOPMENTAL POTENCIES OF THE MESONEPHROS IN *AMBLYSTOMA* IN THE ABSENCE OF THE PRONEPHRIC DUCT

The main purpose of the experiments was to observe the development of the mesonephros in the absence of the pronephric duct. After obstruction of the caudal growth of the pronephric rudiment, embryos were examined at various stages of mesonephric development and a comparison of normal development in the presence of the duct and abnormal development in its absence made at different levels in the mesonephros. Owing to the cephalocaudal differentiation of the mesonephros it was possible to make this comparison at different stages of mesonephric development in a single embryo. To illustrate the findings, sections are taken from an embryo where the caudal growth of the pronephric rudiment on the left side was obstructed by a transplant at stage 22, 25 days before the sections were made. Pl. III, fig. 1 represents a section through the caudal end of the mesonephros and shows the absence of the pronephric duct on the operated side. The development of the mesonephros on the two sides shows no prominent differences—both are in the form of clumps of cells where tubule formation has not yet begun. Nevertheless, examination with an oil-immersion lens shows certain changes in the mesonephric cells of the operated side suggestive of degeneration. Pl. III, fig. 2 is taken through the middle of the mesonephric region of the same embryo, and the normal side shows the beginning of tubule formation. On the operated side, however, there has been no similar advance and the mesonephric cells are still in the form of clumps of cells. A section through the anterior end of the mesonephros (Pl. III, fig. 3) shows an accentuation of this difference. There is an advanced formation of tubules on the normal, unoperated side, but on the operated side there is still no advance beyond clumps of mesonephric cells in which degeneration can be seen.

It is concluded from these observations that, in the absence of the pronephric duct in the species of *Amblystoma* used, the mesonephric cells can differentiate themselves from the surrounding mesoderm and form histologically distinguishable clumps of cells. However, in these circumstances they proceed no further in their development and, in the absence of the pronephric duct, do not form tubules.

RESULTS OF RESECTION OF THE PRONEPHRIC DUCT IN *AMBLYSTOMA*

In the experiments just described the pronephric rudiment is obstructed in its caudal growth so that the pronephric duct does not form caudal to the eighth somite and therefore does not reach the mesonephric region. That is to say, at no stage is there contact between the pronephric duct and the cells of the mesonephros. Another series of experiments were performed which had the object of testing the effect of a temporary contact between the pronephric duct and those cells which would later differentiate into the recognizable cells of the mesonephric units. These experiments were performed on *Amblystoma* at stage 34, which is the stage at which the caudal growth of the pronephric rudiment, forming the pronephric duct, has reached and united with the cloaca. In growing caudally, the pronephric duct grows past the cells of the mesonephros and here lies in contact with them. At the temperatures prevailing when the experiments were performed, forty-eight hours elapsed between the appearance of the pronephric rudiment and its union with the cloaca. Consequently at stage 34 the pronephric duct has been in contact with the cells of the mesonephros for a period not exceeding forty-eight hours.

At stage 34 the pronephric duct was resected in the region of somites nine to fourteen. The method of resection requires some comment, for Howland (1926) records that resection of the duct at this stage is usually followed by complete regeneration. This regeneration was prevented by leaving the wound open for some hours so as to delay healing. As a consequence of this operation the mesonephros in segments 9–14 underwent its development in the absence of the pronephric duct, but, in contrast to previous experiments, after having had a transient contact with it.

Embryos operated upon in this manner were reared for various periods before examination by serial sections. In the operated area it was found that the development of the mesonephros was retarded as compared with the corresponding level on the normal side, but in spite of this retardation the formation of tubules was observed. In an embryo sectioned 20 days after operation a section through the 11th somite (Pl. III, fig. 4) illustrates this point. On the operated side there is absence of the pronephric duct, but there is nevertheless definite evidence of tubule formation although it is not as advanced as on the non-operated side. This represents a striking contrast to the results seen in those experiments where the caudal growth of the pronephric rudiment was prevented. The formation of tubules after duct resection can

proceed to the stage where they become functional, and distended by secretion which cannot escape owing to the absence of the pronephric duct (Pl. III, fig. 5).

These results show that contact between the pronephric duct and the mesonephric cells for less than forty-eight hours results in a definite developmental stimulus being imparted to the mesonephric cells which are thus enabled to form tubules. In the development of the mesonephros in the species of *Amblystoma* used two developmental factors can thus be recognized:

(*a*) A power of differentiation on the part of the mesonephric cells which is independent of the pronephric duct. This power is responsible for the demarcation of mesonephric cells from the surrounding mesoderm.

(*b*) A developmental stimulus due to the pronephric duct which is responsible for the formation of tubules from the mesonephric cells.

COMPARISON BETWEEN THE DEVELOPMENT OF THE MESONEPHROS AND THE OPTIC LENS

In the development of the amphibian optic lens many workers (see Spemann, 1938) have shown a co-operation of developmental forces. As a general conclusion it is stated that the self-differentiating power of the lens ectoderm co-operates with the lens-inducing power of the optic cup. This combination of developmental forces is referred to by German authors as "doppelte Sicherung" of which the accepted English translation is "double assurance". In the development of the mesonephros in the species investigated, the above experiments show that there is a similar combination of developmental forces. First there is a power of differentiation of the mesonephric cells, and secondly there is a stimulating effect due to the contact of these cells with the pronephric duct. Thus it is justifiable to speak of "double assurance" in the case of the development of the mesonephros with the same meaning as in the development of the optic lens. Incidentally it may be pointed out that the term "double assurance" as usually employed is used in a different sense to that intended by the originator. It was originally introduced by Braus (1906) concerning the eruption of the fore-limb in *Bombinator*. This eruption takes place through the gill operculum, and in the eruption two forces were demonstrated by Braus. First, a force due to the expansion and growth of the underlying fore-limb, and secondly some property in the gill operculum, for the foramen through which the limb erupts will form even in the absence of the fore-limb. Here the term "double assurance" implied the co-operation of two factors either of which would produce the same end-result. Spemann uses the term implying the combination of two forces both of which are necessary for complete development. The application of the term "double assurance" to the development of the mesonephros is in the sense of Spemann and not of Braus.

The term "double assurance" has been applied to the development of the mesonephros in the chick and in the anuran *Rana fusca* by Waddington (1937). In both species he found that in the absence of the pronephric duct the mesonephros did not develop beyond clumps of cells. The findings in the Urodele,

Amblystoma, are therefore similar to those in *Rana fusca* and in the chick. This similarity is noteworthy because in the chick the pronephros is a vestigial and non-functional structure, whereas in *Amblystoma* Howland (1921) has shown it to be functional and essential to the life of the embryo.

A comparison between the development of the optic lens and that of the mesonephros has further implications. When the development of the optic lens in different amphibian species is compared, it is found that there is a considerable variation in the relative importance of the inducing power of the optic cup and the self-differentiating power of the lens ectoderm. The expression of this variation is found in experiments in which the developmental potencies of the lens ectoderm is investigated after removal of the optic cup. This serves as a means of investigating the importance of the self-differentiating power of the lens ectoderm in the formation of the completed lens. All gradations are found between those species where, in the absence of the optic cup, there is no development of the lens at all, and those species where the development of the lens is almost complete in these circumstances. That is to say, on the one hand, there are species where lens development is largely dependent on the self-differentiating power of the lens ectoderm, and relatively independent of the inducing power of the optic cup, while on the other hand there are species where the position is reversed. Between these extremes are all intermediate stages. The similarity of the forces concerned in the development of the mesonephros suggests that a similar variation may exist in the relative importance of the self-differentiating power of the mesonephric cells and the stimulating effect due to the pronephric duct, and that this variation may be expressed in the degree of development undergone by the mesonephros of various species of Amphibia in the absence of the pronephric duct. Such information is available from the work of several authors, but it is obtained by a variety of methods and in making a comparison some caution must therefore be exercised. It has been shown above that even a transient contact between the mesonephric cells and the pronephric duct can impart a considerable developmental stimulus to the mesonephric cells. Consequently, in considering experimental results from this point of view only those results can be considered in which care has been taken to see that there is never any contact between the pronephric duct and the cells of the mesonephros. The methods employed can be classified as follows:

(*a*) Prevention of the caudal growth of the pronephric rudiment. To achieve this an obstructing transplant can be used, as in the present series of experiments, or the pronephric rudiment can be removed as soon as it appears and before it has extended caudally into the mesonephric region.

(*b*) Resection of the pronephric duct. This method has been used by Muira (1930) and Shimasaki (1930) but their results are not relevant to the present discussion as the resection was carried out when the pronephric duct had grown into the mesonephric region and therefore had been in contact with the cells of the mesonephros.

(*c*) Transplantation of the presumptive mesonephros into heterotopic situations. Here the only results that can be considered are those in which the mesonephros was transplanted before the pronephric duct had reached the mesonephric region, so that the duct had not been in contact with the cells of the mesonephros. Also, it is necessary that the transplant should have been placed in such a situation that the host pronephric duct was not in contact with it.

The results from the literature that seem pertinent are placed in Table I, which indicates the method employed and includes the results found in the chick. The results obtained for *Pleurodeles waltlii* are from experiments performed by the present writer. Embryos of this species were taken at the stage of the first appearance of the pronephric rudiment, and on the left side the caudal growth of the rudiment was blocked as in *Amblystoma*. A section taken through the middle of the mesonephros 20 days later shows the result obtained. On the operated side, where the duct is absent there are no mesonephric cells at all (Pl. III, fig. 6), in contrast to *Amblystoma* where clumps of mesonephric cells were seen. By examination of all the sections of several embryos under similar circumstances it was possible to confirm that in *P. waltlii*, in the absence of the pronephric duct, the mesonephric cells do not appear at all.

Table I. *The development of the mesonephros in the absence of the pronephric duct*

Species	Author	Method used	Result
Chick	Waddington (1937)	Obstruction of duct	Clumps of mesonephric cells
Chick	Grünwald (1937)	Obstruction of duct	Clumps of mesonephric cells
Rana fusca	Waddington (1937)	Obstruction of duct	Clumps of mesonephric cells
Amblystoma maculatum	Humphrey (1928)	Transplantation	Almost complete development of mesonephros
Amblystoma jeffersonianum	Humphrey (1928)	Transplantation	Almost complete development of mesonephros
Amblystoma (mainly *tigrinum*)	Burns (1934)	Removal of pronephric rudiment	Occasional mesonephric tubule
Amblystoma punctatum	Burns (1938)	Removal of pronephric rudiment	Some mesonephric tubules
Amblystoma ? species	Present paper	Obstruction of duct	Clumps of mesonephric cells
Pleurodeles waltlii	Present paper	Obstruction of duct	No appearance of mesonephros

Examination of this table shows that, in a series of Amphibia, in the absence of the pronephric duct the mesonephric cells undergo a variable development, ranging from no development at all in *Pleurodeles waltlii* to almost complete development in *Amblystoma jeffersonianum* and *A. maculatum*. In *Pleurodeles waltlii*, therefore, the development of the mesonephros is largely,

if not entirely, dependent on the pronephric duct, and the independent power of differentiation of the mesonephric cells is negligible. On the other hand, in *Amblystoma jeffersonianum* and in *A. maculatum* the opposite is the case, and the self-differentiating power of the mesonephric cells is such that it can cause complete development in the absence of the pronephric duct. In species such as *Rana fusca* and the species of *Amblystoma* here investigated, an intermediate state of affairs is present.

It is finally concluded that, in the development of the amphibian mesonephros, the power of independent differentiation of the mesonephric cells co-operates with the stimulating influence of the pronephric duct, but in any one species the relative importance of these two factors cannot be predicted; it can only be discovered by experiments on that particular species.

SUMMARY

1. The developmental potencies of the mesonephros in the absence of the pronephric duct have been tested in *Amblystoma* by obstructing the caudal growth of the duct. It is found that in the absence of the pronephric duct the mesonephros does not develop beyond the formation of clumps of cells, and tubule formation does not take place.

2. By resection of the pronephric duct at appropriate stages of development the developmental potencies of the mesonephros were investigated after a contact of less than forty-eight hours between the cells of the future mesonephros and the pronephric duct. In these cases, in spite of the absence of the pronephric duct, in subsequent development the mesonephros forms tubules and undergoes a more or less complete, but retarded, development.

3. It is therefore concluded that in the development of the amphibian mesonephros two factors operate:

(*a*) A power of differentiation of the mesonephric cells in virtue of which they become demarcated from the surrounding mesoderm.

(*b*) A stimulating influence on the part of the pronephric duct which is necessary for tubule formation.

4. These two factors are compared with the factors operating in the development of the optic lens, and a "double assurance" is suggested for the development of the amphibian mesonephros.

5. Similar experiments performed on *Pleurodeles waltlii* showed that, in the absence of the pronephric duct, mesonephric cells did not become demarcated from the surrounding mesoderm. Comparing these results with those in the literature, it is concluded that in the development of the amphibian mesonephros there is a variation in different species of the relative developmental importance of the differentiating power of the mesonephric cells and the stimulating influence of the pronephric duct.

6. Certain other results of obstructing the caudal growth of the pronephric rudiment are recorded. The development of the pronephros is unaffected, and

also that of the cloaca. There is some evidence to suggest that the cloaca may contribute to the formation of the extreme caudal end of the pronephric duct. The blocked pronephric duct may become dilated or hypertrophied, and in certain circumstances it may make abnormal communications with surrounding structures.

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EXPLANATION OF PLATES I–III

PLATE I

Fig. 1. *Amblystoma*, 30 days' development. Normal development of the mesonephros. Section through the hinder end of the mesonephros. *m*, mesonephros; *d*, pronephric duct. ×150.

Fig. 2. *Amblystoma*, 30 days' development. Normal development of the mesonephros. Section through the middle of the mesonephros. Lettering as in fig. 1. ×150.

Fig. 3. *Amblystoma*, 30 days' development. Normal development of the mesonephros. Section through the anterior end of the mesonephros. Lettering as in fig. 1. ×50.

Fig. 4. *Amblystoma*, experiment of Text-fig. 2. Section in the vicinity of the cloaca. Shows normal right pronephric duct (*r.d.*), and tag of duct-like tissue on the left side (*l.d.*). ×27.

Fig. 5. *Amblystoma*, experiment of Text-fig. 2. Section through the pronephros. The pronephros on the obstructed side (*p.*) shows no abnormality. ×40.

PLATE II

Fig. 1. *Amblystoma*, obstruction to growth of pronephric rudiment. Section immediately cranial to the transplant and shows hypertrophy and dilatation of pronephric duct (*d.*), on the operated side. ×55.

Fig. 2. *Amblystoma*, obstruction of caudal growth of the pronephric rudiment. False communication of pronephric duct (*d.*) with a diverticulum of the gut. ×45.

Fig. 3. *Amblystoma*, obstruction to caudal growth of left pronephric rudiment. Communication of left pronephric duct (*d.*) with the exterior. ×65.

Fig. 4. *Amblystoma*, obstruction to caudal growth of left pronephric rudiment. Communication of left pronephric duct (*d.*), with transplanted cloaca (*c.*). ×40.

Fig. 5. *Amblystoma*, obstruction to caudal growth of left pronephric rudiment. Normal and symmetrical development of cloaca in spite of absence of left pronephric duct. *r.d.* right pronephric duct. ×90.

PLATE III

Fig. 1. *Amblystoma*, obstruction to caudal growth of left pronephric rudiment 25 days previously. Section through the caudal end of the mesonephros. Shows absence of the left pronephric duct and mesonephros approximately of equal development on both sides. *r.d.* right pronephric duct; *r.m.* right mesonephros; *l.m.* left mesonephros. ×110.

Fig. 2. *Amblystoma*, same experiment as fig. 1. Section through the middle of the mesonephros. Shows retarded development of left mesonephros. Lettering as in fig. 1. ×110.

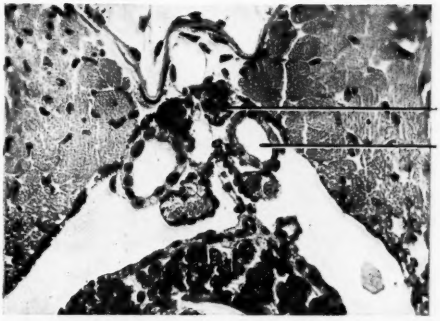
Fig. 3. *Amblystoma*, same experiment as fig. 1. Section through the anterior end of the mesonephros. Shows retarded development and degeneration of left mesonephros. Lettering as in fig. 1. ×110.

Fig. 4. *Amblystoma*. Resection of left pronephric duct in segments 9–13 at stage 34, 20 days previously. Section through the 11th segment. On the left side in spite of the absence of the pronephric duct there is tubule formation in the mesonephros. Lettering as in fig. 1. ×130.

Fig. 5. *Amblystoma*. Resection of the left pronephric duct in segments 9–14 at stage 34, 35 days previously. Section through the 12th segment. On the left side absence of the pronephric duct and distended mesonephric tubules. Lettering as in fig. 1. ×105.

Fig. 6. *Pleurodeles waltlii*, obstruction to caudal growth of left pronephric rudiment 20 days previously. On the left side no appearance of the mesonephros. Lettering as in fig. 1. ×145.

Fig. 1.



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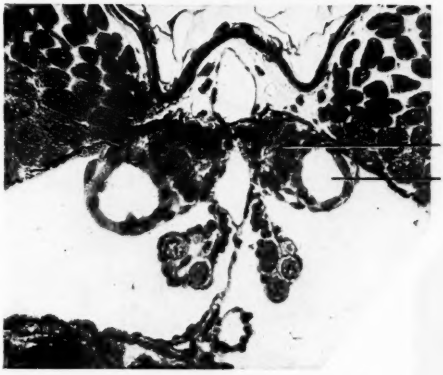

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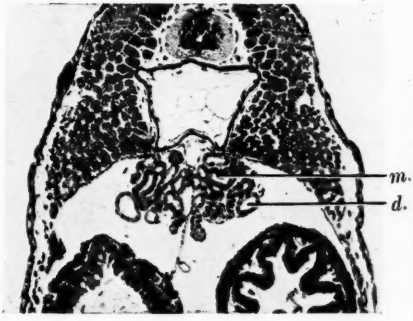
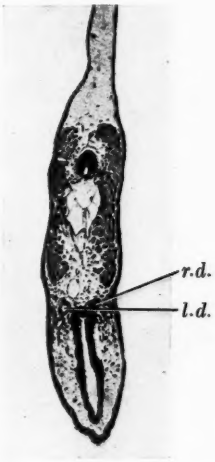


Fig. 4.



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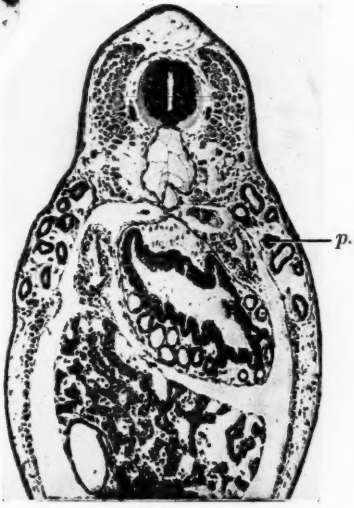

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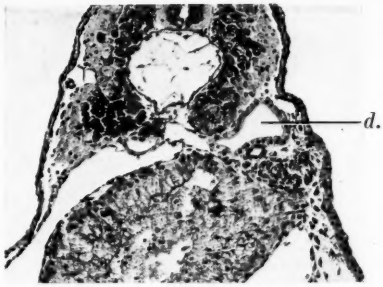

Fig. 1.

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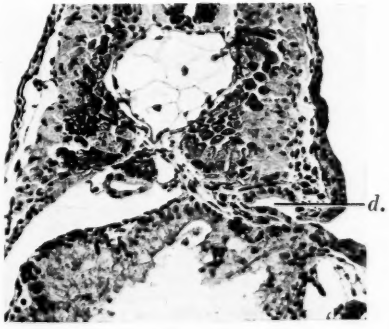

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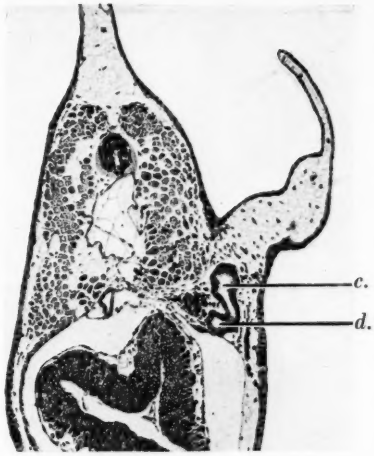

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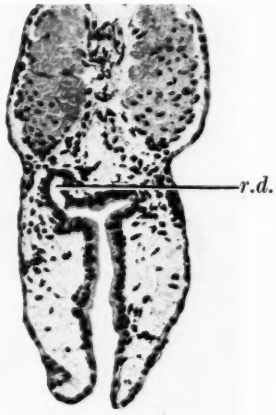

Fig. 5.

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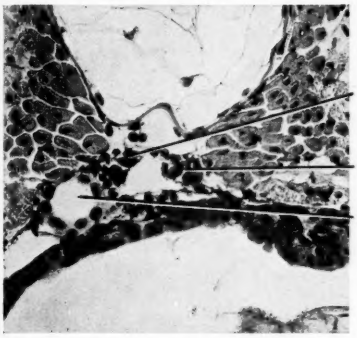
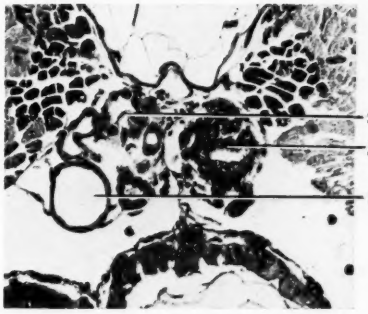


Fig. 1.



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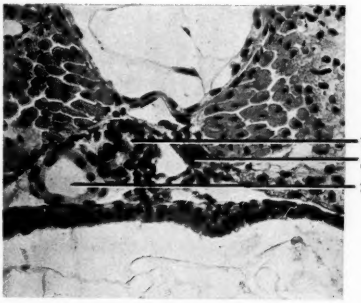

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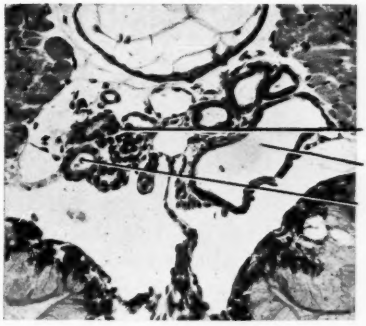

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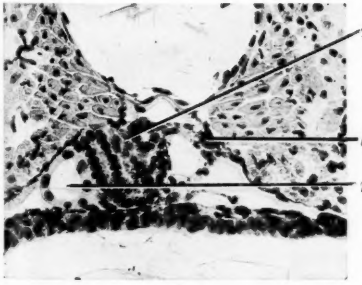

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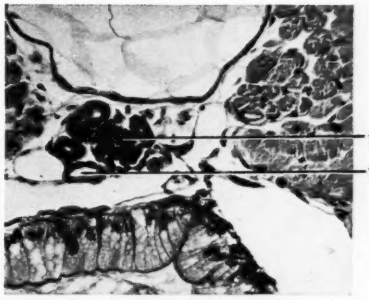

Fig. 6.

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SOME OBSERVATIONS ON THE LYMPHATICS OF THE NASAL MUCOUS MEMBRANE IN THE CAT AND MONKEY[1]

By JOSEPH M. YOFFEY[2] and CECIL K. DRINKER

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In the rabbit, cat, and monkey, experiments have shown that certain dyes and proteins (T-1824, trypan blue, egg albumin, and serum albumin) can be found in cervical lymph following intranasal instillation (Yoffey & Drinker, 1938; Yoffey *et al.* 1938). It was further shown in the rabbit and monkey that vaccinia virus, although it did not immediately enter the lymph stream following intranasal instillation, did so after a preliminary period during which the virus became established in the nasal mucosa; subsequently a steady stream of virus was found entering the blood through the cervical lymph ducts at all times from 12 hr. to 7 days after virus had been dropped into the nose (Yoffey & Sullivan, 1939). It was thought that a histological study of the lymphatics of the nasal mucosa might throw some further light on these observations, and accordingly the present work was undertaken.

For dyes or proteins to reach the lymph stream, they must pass through the intact mucous membrane, a layer of submucous connective tissue, and the endothelium of the lymphatic capillaries. That dyes and proteins can pass through lymphatic endothelium is well established (Drinker & Field, 1933). That protein in small amounts can pass through the intact intestinal mucosa is also usually accepted. Since the first observations of Uhlenhuth (1900) and Ascoli & Viganò (1903) the problem has been investigated by many workers, and extended to cover several kinds of protein. Reviews of the literature are given by Ratner & Gruehl (1934), and Alexander *et al.* (1936). As far as the nasal mucous membrane is concerned, Baum & Trautman (1925–6) described the presence of numerous "stomata" of considerable size, through which the submucous lymphatics were alleged to be in free communication with the exterior. We have been unable to see stomata in freshly fixed preparations, and it seems certain that, when present, they are due to post mortem autolysis at various points in the nasal mucous membrane.

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[2] Fellow of the Rockefeller Foundation, Natural Sciences.

MATERIAL AND METHODS

In several dogs and cats a rich lymphatic plexus was demonstrated, by the method of interstitial injection, in the lateral wall and septum of the nose. These preparations, however, tended to be somewhat patchy. The preparations about to be described were made on one cat and three monkeys (*Macaca mulatta*). In one monkey the posterior pharyngeal wall was exposed 20 min. after death, and at several points interstitial injections were made of small amounts of a 1 : 2 dilution of Higgins' India ink. The pharyngeal wall was then excised and fixed in Zenker, being kept flat by pinning to a cork sheet.

The best injections of the nasal lymphatics were made from the subarachnoid space. In one monkey and one cat under general anaesthesia (intraperitoneal nembutal) a small hole was drilled in the cranial vault and a fine needle inserted into the subarachnoid space. Through this 3·0 c.c. of Higgins' India ink (diluted 1 : 2 with normal saline) were slowly injected. Unfortunately no record of the injection pressure was made, but it was noted that toward the end of the injection increased pressure was necessary, which must have been well above the normal pressure of the cerebrospinal fluid. In a third monkey a similar injection was made of a 0·5 % solution of potassium ferrocyanide and iron ammonium citrate.

After the animals had been killed the head was skinned, cut off, and the mandible and palate removed to allow better access of fixative to the roof of the nose. Fixation was in 10 % formol-saline, to which, in the case of the monkey injected with potassium ferrocyanide and iron ammonium citrate, 1 % of hydrochloric acid was added. After 2 weeks the vault of the skull was sawn off, and also the base of the skull behind the pituitary fossa. The frontal lobes of the brain were dissected away, leaving undisturbed the olfactory bulbs lying on the cribriform plate. The specimens were then returned to fixative for another 2 weeks, after which they were transferred to 5 % nitric acid, in which they were left, with frequent changes, until decalcification was complete. Each specimen was then trimmed so as to leave behind only the olfactory bulbs, the cribriform plate, and the roof of the nasal cavity. In the cat, some of the many laminae in the roof of the nose (ethmoturbinals) were cut off and cleared by the Spalteholtz method. The remainder, and also the blocks of tissue from the two monkeys, were then embedded in celloidin, and serial sections cut at 12μ. Sections were counterstained most effectively with carmine.

LYMPHATICS IN THE POSTERIOR WALL OF THE NASOPHARYNX

Pl. I, fig. 1 is a low-power camera lucida drawing of the mucous membrane from the posterior wall of the nasopharynx of a monkey. The huge size and number of the lymphatics are sufficiently obvious. This heavy distribution of lymphatics is characteristic of the nasopharynx in general, a circumstance doubtless determined by the fact that the nasopharyngeal mucosa suffers from hyperaemia and mild irritation probably more often than any other part of the

body. It is thus constantly flooded with proteinized fluid from the blood capillaries, and this fluid must be carried off by the lymphatics since it will not return directly to the blood capillaries. An analogous situation is presented by the vagina, particularly in monkeys. These animals experience an oedema of the vulvar region which extends up into the vagina at each menstrual period. The oedema fluid is rich in protein, and a complicated and very extensive collection of lymphatics is necessary to remove it (Wislocki, 1939).

LYMPHATICS OF THE SEPTUM AND LATERAL WALL OF THE NOSE

Pl. I, fig. 2 shows the lymphatics in one of the numerous ethmoturbinal laminae in the roof of the cat's nose. The lamina consists of a very thin bony plate with mucous membrane and submucous lymphatics on either side. Looking through the Spalteholtz-cleared lamina the two superimposed layers of lymphatics are therefore seen, a fact which becomes clearer on noting the obliquely cut edge of the lamina, where one layer projects beyond the other. In Pl. I, fig. 3 these laminae are cut at right angles to their surface, and it is evident that the lymphatic plexus is disposed not only in a plane parallel to the mucous membrane, but also at right angles to it, i.e. it possesses depth as well as surface extent. This is brought out more clearly at a higher magnification (Pl. II, fig. 4).

It is significant that the lymphatic vessels are closely applied to the columnar epithelium, with usually only a very thin layer of subepithelial connective tissue intervening. Dyes, proteins, viruses, or bacteria must therefore come into contact with the submucous lymphatics as soon as they pass through the epithelium. It can thus be readily understood why it is that vaccinia virus, from a primary focus in the mucous membrane, can so constantly be found in cervical lymph (Yoffey & Sullivan, 1939). Similar considerations apply to the finding in cervical lymph (rabbit) of type III pneumococci after nasal instillation (Schulz *et al.* 1938). It is possible that the virus of the common cold disseminates from the nasal mucosa in the same way.

Pl. I, fig. 3 and Pl. II, fig. 5 are from the cat and monkey, respectively, in which India ink was injected into the subarachnoid space. The sections pass through olfactory bulb, cribriform plate, and roof of nose, the olfactory bulb being cut at right angles to its long axis. In each specimen olfactory nerve bundles may be seen passing through foramina in the cribriform plate; particles of India ink are also passing through the same foramina, surrounding the nerves. In the cat (Pl. I, fig. 3) there is present, immediately below the cribriform plate and on either side of the lamina perpendicularis of the ethmoid, a dense accumulation of India ink in the tissues, suggesting a sudden extravasation; from this focal accumulation the ink passes freely into the submucous lymphatics. As far as we can make out in these sections (cat) there is perfectly free communication between the perineural space and the connective tissue spaces below the cribriform plate. This apparently free communication may

have been artificially produced by the injection pressure rupturing a thin perineural sheath. Unfortunately there is such a dense mass of ink that intimate structural details are difficult to make out.

In the monkey (Pl. II, fig. 5) ink does not seem to have passed through the cribriform plate quite so freely, but again the perineural location of the ink is perfectly evident (Pl. II, fig. 6). In this particular preparation (Pl. II, fig. 5) the mucous membrane of the roof of the nose has come away. In the monkey injected with potassium ferrocyanide and iron ammonium citrate the mucous membrane remained *in situ*, and a well-marked submucous lymphatic plexus was present, exactly like that figured by Weed (1914, Pl. IV, fig. 8).

DISCUSSION

The method employed in the preparation of the specimens depicted in Pl. I, figs. 2, 3, Pl. II, figs. 4–6, while devised primarily for the purpose of injecting the nasal lymphatics, raises once again the question of the precise nature of the communication between these lymphatics and the subarachnoid space—a question of great theoretical and practical interest. It seems to be unquestionable that, after injection into the subarachnoid space, simple or colloidal solutions, as well as suspensions of particulate matter, may readily be forced down the cranial nerves between the nerve sheaths (which are continuous with the membranes reflected from the brain) and the nerve fibres. In the present instance it is the olfactory nerve with which we are primarily concerned. Increased injection pressure, as in the present experiments, may force down much more fluid, but apparently some comes down even when pressures are normal. Weed (1914) showed that if he made subarachnoid injections leading to the deposition of granules of Prussian blue, marked deposits of the dye accumulated in the perineural spaces about the olfactory nerves, and great masses of it in the subarachnoid cul-de-sac just above the cribriform plate. If the original ferrocyanide mixture used by Weed was injected into the subarachnoid space under high pressure, fluid could be made to pass out through this olfactory pathway so rapidly as to drip from the nose. When a dye, such as trypan blue, is injected into the subarachnoid space with just enough pressure to get it in, after a short time—15–30 min.—one may observe that not only has the mucous membrane of the olfactory area become very blue, but that the colour also extends down the deep lymphatics and through the cervical lymph nodes. It is a dramatic demonstration of the ease with which the cervical lymph pathway can be injected from the inside of the cranium.

The intimate details of what happens are apparently as follows. Dye or India ink injected in the subarachnoid space passes downward along the outside of the nerve, separating off the nerve sheath, until eventually, if undue pressure is employed, it may track along the nerve as far as the mucous membrane, to be forced out at the surface of the mucosa at points of emergence of olfactory nerve terminals; or it may break through the sheath and fill the meshes of the loose connective tissue of the submucosa. This apparently is what has happened

in the cat preparation (Pl. I, fig. 3). Once in the submucous connective tissue, dye or India ink promptly enters the very numerous and large lymphatics which characterize the entire nasopharyngeal mucous membrane and, *via* these channels, soon reaches the large cervical lymphatic trunks.

As the nerve passes through the dura mater and arachnoid, there runs beside it an extension of the subarachnoid space which gradually narrows as the dura and arachnoid come closer to the pial covering of the nerve, with which they blend outside the skull to form the perineural sheath. The prolongation of the subarachnoid space which extends down between the dura and arachnoid on the one hand, and the nerve with its pial investment on the other, acts as a fluid wedge when the pressure in the subarachnoid space is increased. Presumably this wedge first strips the progressively thinning dura-arachnoid away from the nerve, and ultimately ruptures it. It is clear, however, that in the absence of such a fluid wedge extending between the dura-arachnoid and the nerve, a communication between the nasal lymphatics and the subarachnoid space would be difficult to demonstrate, and this fact is brought out most strikingly when one considers the passage of substances in the reverse direction, i.e. from the nose to the interior of the cranium.

Experimental evidence indicates that the upward path into the brain case (leaving out of consideration living particles) is traversed only with the greatest difficulty. In fact, apart from simple crystalloids (Le Gros Clark, 1929; Faber, 1937–8), it has not been shown to occur. Thus Le Gros Clark (1929) left a 0·5 % solution of trypan blue in the nose of a rabbit for 24 hr., and at the end of that time found no sign of dye within the cranium. Our own experience with trypan blue and T-1824 has been similar (Yoffey & Drinker, 1938). In no case was dye ever found inside the cranium of cats, dogs, or rabbits after it had been left in the nose for several hours. These experiments were all the more convincing since the cervical lymph ducts were cannulated at the same time, and it was therefore possible to show that dye was present in the lymph in high concentration over many hours. Evidently, then, dye can readily pass from subarachnoid space to nasal lymphatics, but not in the opposite direction. How is this one-way passage ensured?

Any tendency for fluid which has penetrated the nasal mucosa to enter the perineural channels is apparently obviated by the fact that the neural sheath fits tightly against the nerve filaments, and even if pressure could be applied, it would be almost impossible to force anything in; the only effect would be to push the sheath more tightly against the nerve. But it seems unlikely that this sleeve valve would ever be subjected to great pressures; before a high pressure could be attained, fluid in the submucous connective tissue spaces would drain off into the submucous lymphatics.

There is a further point to be considered. The cerebrospinal fluid is at a considerably higher pressure than cervical lymph. Thus Weed & Hughson (1921–2) found in seventy-seven cats under ether an average cerebrospinal fluid pressure of 119 mm.; this pressure underwent only slight fluctuations, e.g.

11 mm. in an animal observed for 2 hr. As to cervical lymph, McCarrell (1939*b*) found in six dogs pressures ranging from −28 to 32 mm. of water, the negative pressure which sometimes develops being due to suction of lymph into the great veins. Cerebrospinal fluid, therefore, is at about three or more times the pressure of cervical lymph; it is presumably this pressure gradient which accounts for the fact that some cerebrospinal fluid normally drains away into the lymph. According to Weed (1922) "the absorption of the cerebrospinal fluid is a twofold process, being chiefly a rapid drainage into the great dural sinuses, and in small part a slow indirect escape into the true lymphatic vessels". If there is a slight but continuous seepage of fluid through the cribriform plate into the nasal lymphatics, dye which had penetrated the mucosa would be swept on into the lymph vessels rather than pass upwards into the interior of the cranium. However, it seems unlikely that this current can be very large, and that much cerebrospinal fluid leaves the subarachnoid space in this way. For one would have to explain the passage of simple solutions (Le Gros Clark, 1929; Faber, 1937–8) from the nose to the interior of the cranium on the basis of diffusion, which it would be difficult to visualize if there were a copious flow of cerebrospinal fluid away from the brain.

The argument thus far has been based on the generally accepted view that there is a complete and uninterrupted perineural sheath. If it should turn out that the perineural sheath is incomplete, then the passage through it of cerebrospinal fluid would offer no difficulty; the failure of dyes to pass from the nose into the cranium would have to be explained on the grounds that their diffusion rate is less than that of the outward current of cerebrospinal fluid. If, however, the perineural sheath is complete, one must assume that it thins down sufficiently to allow cerebrospinal fluid to pass through; its thickness and physical properties must be very like those of capillary endothelium. In other words, it must function as a semipermeable membrane through which colloidal dyes can pass from the subarachnoid space downwards aided by the pressure of the cerebrospinal fluid, but cannot pass through from below upwards against this pressure. Crystalloids such as potassium ferrocyanide and iron ammonium citrate could pass through in either direction.

A thin perineural sheath could readily be ruptured, a possibility which has already been mentioned in discussing the apparently free communication between the perineural spaces and the submucous connective tissue spaces in the cat (Pl. I, fig. 3). The evidence of the present experiments is not sufficient to enable one to decide definitely whether the perineural sheath is normally complete or not. If the perineural spaces open freely into the submucous connective tissue of the nose, one would expect a measurable amount of cerebrospinal fluid to drain away normally by this route, the amount being appreciably increased if the pressure of cerebrospinal fluid is raised. Since lymph from the nose drains finally into the cervical lymph duct (Yoffey & Drinker, 1938) this cerebrospinal fluid should give rise to a free and spontaneous flow of cervical lymph.

Previous experiments have shown that a spontaneous flow of cervical lymph does occur with fair regularity in the monkey, though not so frequently in the cat. On the other hand, a comparison of Pl. I, fig. 3 and Pl. II, fig. 5 would lead one to anticipate just the opposite result; for judging by the escape of India ink from the perineural spaces, the quantity of cerebrospinal fluid draining into the nasal lymphatics should be greater in the cat. It has already been emphasized that in view of the increased injection pressures used, these findings should be interpreted with caution. More accurate information might well be obtained by the technique of McCarrell (1939*a*), obtaining first a steady flow of cervical lymph, and then noting the effects upon this lymph flow of experimentally induced changes in the pressure of the cerebrospinal fluid.

SUMMARY

1. In the nose and pharynx of the cat and monkey there is a rich lymphatic plexus which drains into the deep cervical lymph duct.

2. The vessels of this plexus are in very close contact with the nasopharyngeal epithelium.

3. The plexus may readily be injected from the cranial subarachnoid space with India ink, or a solution of potassium ferrocyanide and iron ammonium citrate.

4. There is a brief discussion of the permeability of the perineural sheath, and the precise mode of communication between the subarachnoid space and the nasal lymphatics.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Camera lucida drawing of a section through the posterior wall of the nasopharynx of a monkey. Note the numerous large lymphatic channels, filled with India ink, close to the epithelium.

Fig. 2. Portion of one of the ethmoturbinal laminae of a cat, in which India ink was injected into the cranial subarachnoid space. The lamina has been cleared by the Spalteholtz method, so that viewed by transmitted light the submucous lymphatic plexus is seen on both sides. At the left and lower borders the lamina has been cut obliquely, and the plexus on one side is seen projecting beyond that on the other. ×22.

Fig. 3. Section through olfactory bulb, cribriform plate, and roof of nose, in a cat in which India ink was injected into the cranial subarachnoid space. Note ink passing through foramina in cribriform plate, on either side of the lamina perpendicularis of the ethmoid. Note also the dense masses of ink immediately below the cribriform plate, and the widespread filling of the submucous lymphatic plexus. *A*, olfactory bulb; *B*, cribriform plate; *C*, fibres of olfactory nerve and India ink passing through cribriform plate; *D*, nasal cavity; *E*, ethmoturbinal lamina; *F*, nasal septum. ×7.

PLATE II

Fig. 4. Portion of Pl. I, fig. 3 at a higher magnification. Note the lymphatic plexus immediately adjoining the columnar epithelium. ×25.

Fig. 5. Section through the olfactory bulb, cribriform plate, and roof of nose, in a monkey which had received an injection of India ink into the cranial subarachnoid space. Compared with the cat (Pl. I, fig. 3) the ink appears to have come down in smaller quantities. The mucous membrane of the roof of the nose has come away during the preparation. *A*, olfactory bulb; *C*, olfactory nerve bundle surrounded by India ink passing through a foramen in the cribriform plate; *D*, roof of nasal cavity; *M*, marrow cavity in upper end of nasal septum. ×7.

Fig. 6. Portion of Pl. II, fig. 5 at higher magnification. Same lettering. Note the ink tracking down in the perineural space. ×25.

Fig. 1.

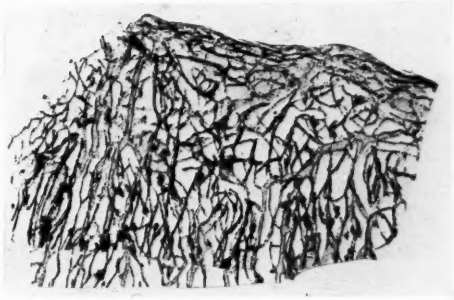

Fig. 2.

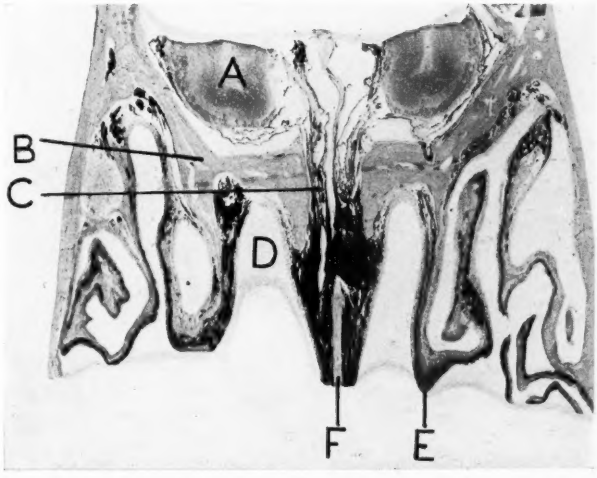

Fig. 3.

YOFFEY AND DRINKER—LYMPHATICS OF THE NASAL MUCOUS MEMBRANE

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Fig. 4.

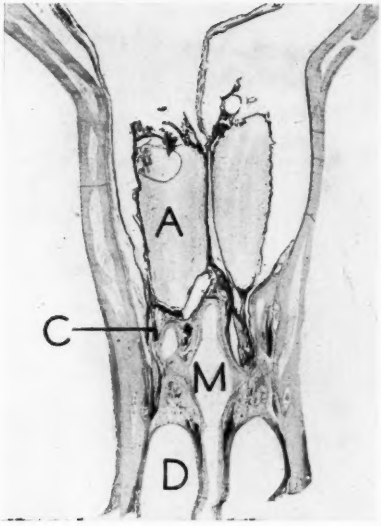

Fig. 5.

Fig. 6.

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THE INNERVATION OF THE GUM OF *TALPA EUROPEA*

By W. LEWINSKY and D. STEWART

From the Department of Anatomy, University of Manchester

In a recent paper we have given an account of the innervation of the human gum (Lewinsky & Stewart, 1938). Since then, using the same technique, the opportunity has been taken of studying the innervation of this tissue in several of the mammalian orders, and it is our intention at a later date to give a complete account of this investigation. However, as the nerve supply of the gum of the mole is so interesting, it was decided to publish a separate description of our findings in this animal.

The gum of the mole has an unusually ample nerve supply. Botezat (1907) found a similar condition in the tissues of the hard palate in the same animal. The nerve supply in the interdental papillae is particularly rich. In serial longitudinal sections of the jaw, with the gum and teeth attached, large nerve bundles could be seen which first of all lay immediately over the periosteum of the jaw; they then become more superficial and pass into the subepithelial tissue of the gum. In this region the fibres become thinner and still more numerous, and some of them form a plexus. Other fibres enter directly into the epithelium. In this region the arcade-like distribution of the nerves, which we have already described in the human gum, can again be seen. These arcades consist of nerve fibres running along the border-line between the epithelium and the papillae. Intra-epithelial fibres arise from these arcades. We have also observed that in the subepithelial tissue some of the nerve fibres run in close contact with the blood vessels and possibly terminate in them.

The general distribution and abundant nerve supply of the subepithelial tissue is demonstrated in Pl. I, fig. 1. In the deeper tissues, many large bundles will be observed. One of these bundles ascends towards the epithelium and supplies fibres to that tissue, which divide into finer branches, some of them terminating in end-organs while others appear to have free endings.

Under a higher magnification, a subepithelial plexus is seen to be formed by ascending bundles. It is also possible to distinguish that, in the gum of the mole, this plexus is formed by two types of fibres, namely, thick myelinated fibres and thin fibres which are often non-myelinated.

An arrangement of the nerve bundles in the subepithelial tissue has been seen which appears to be characteristic for the mole (Pl. I, fig. 2). On approaching the epithelium, the fibres spread out and form a leash, some of these fibres passing on into the epithelium. This leash formation has been found in a

large number of sections in different parts of the gum; it does not seem to occur in the gums of members of any other mammalian order which we have examined.

Three types of intra-epithelial endings are found in the gum of the mole: (1) free nerve endings, (2) end-swellings, (3) touch menisci and Merkel's cells.

The free endings have already been mentioned. An example of an end-swelling is shown in Pl. I, fig. 3. A nerve fibre can here be seen running towards the superficial layers of the epithelium and ending in a rather roughened elongated swelling. In the adjoining sections, there is no sign of any further branches or fibres connected with the swelling. Botezat mentions that he found very rarely club-like bodies of a simple nature in the palate of the mole. We believe that this elongated swelling is of a similar nature to the end-bodies of Botezat.

The third type of ending forms the most important part of this investigation. The problem of the Merkel touch-cells has interested anatomists since 1880, when Merkel gave an account of the touch-cells in the snout and hard palate of the mole. He considered that these cells are differentiated from the other epithelial cells by having a light globular structure and a light nucleus, and he believed that they are terminal ganglion cells. He was of the opinion that the protoplasm of the cells continues into the nerve fibre, whereas the membrane of the cell is continuous with the nervous sheath. In the same year, Ranvier (1880*b*) described the connexions of the nerve fibres with epithelial cells as follows: axon cylinders form touch menisci on the surface of the cell, and the menisci are connected with each other by thin fibres. These touch-cells and menisci were described also by Sczymonowicz (1895), Botezat (1901, 1902, 1907 and others), Dogiel (1905), and Bielschowsky (1907) and others. They were mainly seen in the snout and hard palate of the mole, but were also found in the snout of the pig and dog, and in tactile hairs. Up to the present, they have not been described in the gum. They were always found to be situated in the deeper layer of the epithelium, and the finer structure of these tactile discs is supposed to consist of a neuro-fibrillar net (Dogiel & Botezat). There is a certain dissensus of opinion as to whether the Merkel touch-cells are of different appearance and size from the other epithelial cells, and whether the touch menisci are situated inside or on the surface of the cell. More modern descriptions are given by Kadanoff (1928) of the Merkel-Ranvier touch menisci in the snout of the pig. He described the touch menisci as oblong neuro-fibrillar nets, and in his drawing the touch-cells appear lightly stained and much bigger than the ordinary epithelial cells, occupying the space of about two or three epithelial cells, whereas in the human gum and epiglottis he found only neuro-fibrillar nets without Merkel cells. Boeke (1932) published a very beautiful drawing showing tactile discs from the root sheath of the tactile hair of the mouse (after a drawing by Akkeringa). He came to the conclusion that the neuro-fibrillar disc does not lie between the cells with a somewhat differentiated epithelial cell covering it, but that it is situated within the proto-

plasm of the differentiated cell itself. He gives a very interesting and detailed description of its finer features, but mentions that the tactile disc will require much further study before its structure can be elucidated.

As far as we are aware, the Merkel-Ranvier touch menisci have not been photographed before, and this we believe to be one of the reasons for some of the difficulties which have arisen in harmonizing the different results which various investigators have obtained.

Pl. I, fig. 4 shows a fibre arising from a fairly thick subepithelial trunk; it runs into the epithelium and gives off branches which end in tactile menisci which are not very clearly seen in this figure. The shape of the menisci are clearly shown in Pl. I, fig. 5 which is of a much higher magnification. The semi-lunar shape is now clearly visible and within the hollow of the cup lies a Merkel's touch-cell which in this specimen does not appear to differ in size or staining properties from the other epithelial cells. The touch meniscus appears as a homogeneous body without any special internal structure. This however is not the condition which is actually present, for if a touch meniscus be viewed tangentially (Pl. I, fig. 6), it will be seen that it is formed of a definite neuro-fibrillar network.

It was mentioned above that in one specimen the epithelial touch-cell did not differ from the other epithelial cells, and this has been the general rule in a large number of sections which have been examined. One exception has been seen and this occurs in Pl. I, fig. 6 where there is a touch-cell which is definitely larger and stains more deeply than the other cells. This however is quite exceptional in these specimens.

The present series of slides were cut at 12μ and therefore do not supply adequate material to determine whether the touch menisci lie external to or within the Merkel's touch-cells as suggested by Boeke. It can only be said that there was no evidence in favour of their having an intracellular position.

In our investigations into the innervation of the periodontal membrane, we found that the nerve-endings vary from order to order and that there seem to be specific types of nerve-endings in each order. The hypothesis therefore suggested itself that in other tissues of the body the type of nerve-ending might also vary in the different orders. Our latest investigation supports that hypothesis, for it is quite clear that there is a very marked difference in the innervation of the gum of the mole and the human gum. In the human gum the nerve-endings have the form of coils. The intra-epithelial fibres are relatively few and Merkel-Ranvier touch menisci are never found. Also, the breaking up of the bundles into leashes is not seen.

In the extensive literature on peripheral nerve-endings, expressions of regret will be found that authors have been unable to find structures in a tissue which have been described by other workers in similar tissues of another animal. It is always assumed that they ought to be present and that some fault in the histological technique was the cause of their absence. If, however, the hypothesis which we have enunciated is correct, it is much more probable that

the nerve-endings were not seen because they did not exist in that particular tissue.

SUMMARY

1. The gum of the mole has a much richer nerve supply than the human.

2. The nerves show three types of ending: free endings, end swellings and touch menisci.

3. An account is given of the structure of the touch menisci and their relation to Merkel touch-cells.

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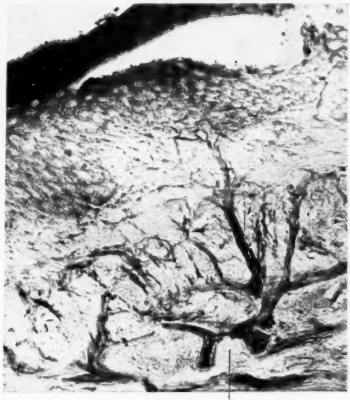

Fig. 1.

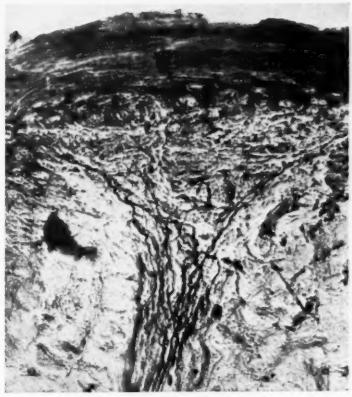

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6

LEWINSKY AND STEWART—THE INNERVATION OF THE GUM OF *TALPA EUROPEA*

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THE CERVICAL REGION OF THE LACERTILIA

A CRITICAL REVIEW OF CERTAIN ASPECTS OF ITS ANATOMY

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Anatomy Department, University of Leeds

We owe our present conception of the cervical anatomy of the Lacertilia, and more especially our knowledge of the morphological significance of the associated pharyngeal derivatives, principally to researches carried out during the past fifty years. But whereas, in this field, the names of Continental observers figure prominently, there has been no significant contribution on the part of any British investigator. However regrettable this may be, it would occasion no concern, if it were not for the prevailing tendency, in the English literature, to ignore, in greater or less degree, the valuable Continental investigations, and to rely rather on the few and inadequate papers which have appeared in the English journals. This absence of any satisfactory account in English would seem to be responsible for many of the misstatements which continue to appear. To disregard facts which are well established, and homologies which are now accepted by most authorities, is to ignore the progress which has been made during the last fifty years. These advances form, at the present time, the only rational basis from which any systematic research on this subject can proceed, and ignorance of them can only result once more in the confusion which existed many years ago.

English-speaking anatomists appear to have restricted their observations on the cervical region of the Lacertilia almost entirely to the vascular system. It is true that, in this connexion, there have been several notable contributions which in themselves provide adequate testimony to their value (O'Donoghue, 1920; Bhatia & Dyal, 1933). But when we turn to another important aspect of cervical anatomy—the epithelial pharyngeal derivatives—the situation is quite the reverse. On this subject we have made no contribution to the great and significant advances which have been such a feature of the last half century. A search of the English literature reveals very few papers which deal in any way with the pharyngeal derivatives of the Lacertilia. As long ago as 1844, Simon gave a very brief but quite accurate account of the thyroid in the Sauria, and he was, in fact, the first to distinguish correctly the thyroid from the thymus. Misidentification of these two structures, however, was quite common even after that date, and by such an eminent observer as Rathke. Handfield-Jones (1849) refers to the thymus and the thyroid in the Reptilia, but gives no specific account of the conditions in the Lacertilia. Watney (1882) also studied the reptilian thymus in some detail, but did not examine any lacertilian. More recently, Mrs F. D. Thompson (1911) has described the

thyroid and parathyroids in the vertebrate series. However accurate and complete this account may be for other classes of vertebrates, it is certainly inadequate as far as the Reptilia are concerned, and in this respect can be, and has been, severely criticized (Trinci, 1912). Although Thompson states that she dissected a lacertilian (*Anolis velifer*) no account of it is given. Only one figure illustrates her description of the features in the Reptilia as a whole, and there is not a single reference to the literature which then existed, although, even at that date, many important papers had already appeared. In spite of its deficiencies, her account for the Reptilia appears, nevertheless, to have been accepted quite unconditionally by several writers (Swale Vincent, 1924; Marine, 1932). There is, however, one interesting point in her description of the reptilian "parathyroid", the significance of which has apparently escaped notice. This relates to the arrangement of the epithelial cells into acini ("secretory tubules") or vesicles, with a very small lumen (p. 104), and will be referred to later.

As there would appear to be ample justification for a restatement of what is known of the cervical region of the Lacertilia, I propose, therefore, to summarize the present viewpoint, with special reference to *Lacerta*, several specimens of which I have examined by dissection and by serial sections. In view of its phylogenetic interest, and also because I have had the opportunity of dissecting several specimens, certain features of the cervical anatomy of *Sphenodon* will be discussed. As van Bemmelen has indicated, *Sphenodon* shows a striking resemblance to the Lacertilia in the organization of its cervical region, and he has in fact regarded it as the basic saurian type in his monograph on the cervical region of reptiles (1888).

It would be out of place here to attempt a complete bibliography, such as may be found in numerous works (Kohn, 1899; Hammar, 1911; Trinci, 1912; Pischinger, 1937). Since a large number of the papers relative to this subject are more or less controversial, I have restricted the bibliography only to those works which I consider significant.

THE THYROID GLAND

Developmentally and histologically, the thyroid gland in the Lacertilia and in *Sphenodon* appears to be identical with that in other amniotes, although Cowdry (1922, p. 213) states that the gland is more highly developed in the higher vertebrates. It is probable that this refers to macroscopic features, as there is no evidence that it is so histologically. The gland is composed of vesicles, of varying dimensions, lined by a simple cuboidal epithelium, and containing colloid. It is invested by a thin collagenous connective tissue sheath which is continuous with the rather sparse intervesicular connective tissue in the interior of the gland.

In *Lacerta* (Figs. 1, 2) the thyroid lies under cover of the muscles constrictor colli, episterno-hyoideus superficialis and profundus (synonyms after Edgeworth, 1935). It is situated immediately ventrally to the trachea and related

structures (arteria laryngo-trachealis and inferior laryngeal nerves on both sides, and the vena trachealis on the right side). It is often described as lying immediately in front of the pericardium (Swale Vincent, 1924), but this is not necessarily always the case, for it may be, as in *Lacerta*, somewhat more rostrally placed; however, even in *Lacerta*, there is considerable individual variation. In most reptiles, the thyroid is a comparatively simple elongated organ, disposed transversely across the trachea, to which it is loosely attached by

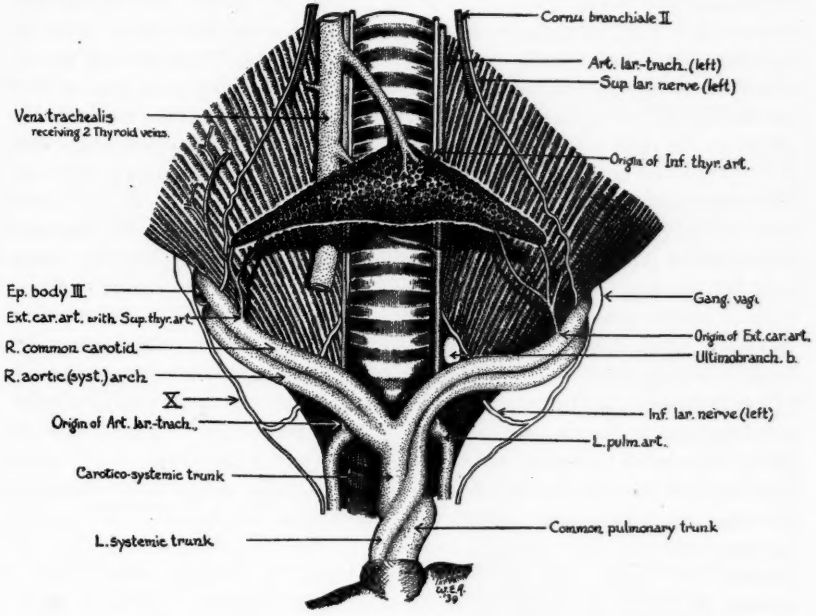

Fig. 1. *Lacerta*. The thyroid apparatus seen from the ventral aspect after removal of the constrictor colli muscle, the infrahyoid muscles, and the pectoral girdle. The ultimobranchial body normally lies slightly more caudally than is shown, being usually largely hidden by the left common carotid artery.

connective tissue. According to Krause (1922) it lies within a cervical lymph-sinus. It is usually relatively straight (*Lacerta*, *Sphenodon*) but, again, it may be curved as in *Monitor*. It is generally largest at its middle and tapers to its lateral extremities (*Lacerta*, Fig. 1), but not uncommonly the reverse may be seen—two lateral "lobes" with an intermediate "isthmus" (*Anguis*). More rarely still the thyroid may be completely separated into two halves, one on either side of the trachea. The statement made by Thompson (1911) and Swale Vincent (1924) that the last-mentioned is the most common type is, therefore, contrary to fact.

In the Lacertilia and in *Sphenodon* the gland has invariably a double arterial supply on each side (van Bemmelen, 1887, 1888; O'Donoghue, 1920).

(1) *Superior thyroid artery* (Figs. 1, 2). This is the first branch of the external carotid artery. As it approaches the lateral extremity of the gland it usually divides into two branches both of which pass to the lateral part of the gland.

(2) *Inferior thyroid artery* (Figs. 1, 2). This arises, curiously enough, from a branch (arteria laryngo-trachealis) of the pulmonary artery. Just beyond the truncus arteriosus, at the summit of its arch, each pulmonary artery gives off an arteria laryngo-trachealis (see Wyeth, 1924, Fig. 16). These ascend, one on either side of the trachea, to the larynx (Figs. 1, 2). For the greater part of their course they are accompanied by the inferior laryngeal nerves (seu recurrentes); while, on the right side, the arteria laryngo-trachealis has also in relation with it the vena trachealis. Although both arteries are usually present, they are often unequal in size; one, however, may occasionally be absent, in which case there is only one inferior thyroid artery, passing as usual to the posterior surface of the intermediate part of the gland. Not uncommonly, too, even when both laryngo-tracheal arteries are present, we may still find only one inferior thyroid artery, arising from the larger. I cannot subscribe to the assertion of Bhatia & Dyal (1933), who after describing these vessels (which they call tracheo-laryngeal arteries) in *Hemidactylus* state (p. 436) that "among Lacertilians *Sphenodon* is the only example in which the tracheo-laryngeal arteries are described to have a similar origin from the pulmonary". Van Bemmelen (1888) has stated that they can be seen always in the Lacertilia, and I can at least confirm this for *Lacerta*. The significance of this unique blood supply is discussed by O'Donoghue (1920) who emphasizes the fact that the arteria laryngo-trachealis also supplies branches to the oesophagus, and thus represents the vestige, in the Lacertilia, of the large oesophageal artery which is present in certain Amphibia where the respiratory function is either partly or wholly effected by the mucosa of the buccal cavity and pharynx. In some forms, indeed, the pulmonary arteries may be absent and their place taken entirely by these oesophageal arteries. It is interesting also to correlate the presence of this inferior thyroid artery with the correspondence which exists between thyroid and lung tissue, a fact which has been stressed by Marine (1922, p. 270).

The thyroid veins (usually two in number) open into the vena trachealis which, in *Lacerta*, is present only on the right side (Figs. 1, 2, 4), but in *Sphenodon* is bilateral. O'Donoghue (1920) has emphasized the fact that the vena trachealis must not be regarded as the vena jugularis externa, a mistake, however, which is still commonly made.

The nerve supply of the thyroid appears to be derived entirely from the inferior ("recurrent") laryngeal nerves. Under a dissecting microscope several minute branches can be seen passing to the posterior surface of the gland in

company with the inferior thyroid artery (Fig. 2). I was not able to identify any branches from the superior laryngeal nerves, which pass forward close to the lateral poles of the gland (Fig. 1).

Quite recently, Bourne (1935) has stated that, in the lizard *Egernia kingii*, "the thyroid differs from any previously known thyroid gland in any class of vertebrates". Such a dogmatic assertion is at once suspect when we find, on consulting his references, that he has relied for his information solely on two very inadequate sources—Owen (1866) and Swale Vincent (1924)—while he

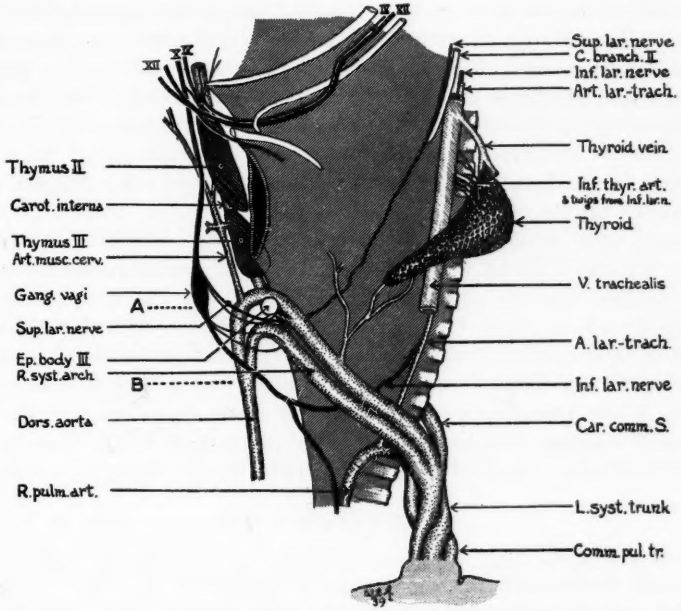

Fig. 2. *Lacerta*. A semidiagrammatic view of the cervical region, right lateral aspect. The internal jugular vein, which overlies the thymus glands, has been removed, the ganglion of the vagus is displaced dorsally, and the recessus piriformis has been cut away to expose the structures passing behind it. A and B represent the levels of section in Figs. 4 and 5.

has completely ignored not only the excellent figures of van Bemmelen (1888) but also the numerous important researches by other workers in this field. In his description, he makes no mention of the inferior thyroid arteries of which, indeed, he could have had no knowledge (but whose absence would be unusual in a lacertilian); apart from this, however, there is no reason to believe that the thyroid gland of *Egernia* is not typically lacertilian. It certainly appears to present no real or salient difference from that of any other genus of this order, or if it does, no convincing evidence of it has been presented. Furthermore, there are certain other statements in his paper which cannot readily be

accepted. He states (p. 516) that "from the ventricle were given off two pairs of vessels, the right and left aortae and common carotids". Such an arrangement would be so unusual in a lacertilian (or indeed in any reptile) that I cannot believe that it accurately represents the facts, while the omission of any reference to the common pulmonary artery, the largest component of the truncus arteriosus, is in itself quite unjustifiable, particularly in view of the fact, mentioned above, that the pulmonary arteries contribute to the arterial supply of the thyroid gland. Also, the nomenclature which has been adopted in the description of the venous arrangements is quite unsatisfactory. Again, in describing the nerve supply of the thyroid in *Egernia*, Bourne states that it takes the form of "two fine branches from the laryngeal nerves of either side". In the Lacertilia, surely, the laryngeal nerves on each side are always paired—the superior and inferior laryngeal nerves, both of which arise from the vagus. The inferior is usually called the "recurrent" but it is no more deserving of this name than is the superior which, owing to the persistence of the ductus caroticus in the Lacertilia, at first descends and then turns forward again around the carotid arch (Fig. 2), just as the inferior does around the aortic (systemic) arch. Since, then, the "laryngeal nerves" mentioned by Bourne appear from his Text-fig. 2 to correspond to the inferior laryngeal nerves, are we to assume that the superior laryngeal nerves are absent in this genus? Finally, even a superficial familiarity with the literature would have obviated the quite unnecessary examination of "a mass of connective tissue...near the heart" in the hope of disclosing the presence of thymus or "parathyroid" tissue, because in the Lacertilia these structures do not occupy this position. They are, on the contrary, situated laterally on the pharyngeal wall, in close relationship with the great vessels of the neck, and quite removed from the pericardium.

THE THYMUS GLAND

Most authorities agree that the definitive thymus in the Lacertilia, and in *Sphenodon*, is derived from the dorsal parts of the second and third pharyngeal pouches (van Bemmelen, 1886; de Meuron, 1886; Maurer, 1899; Saint-Remy & Prenant, 1903; Wyeth, 1924; Brachet, 1935). In the earlier stages of development, however, other pouches may produce transient masses of thymus tissue, which usually regress but which occasionally persist.

In the adult lacertilian, and in *Sphenodon*, therefore, the thymus almost invariably consists, on either side, of two separate "lobes", lying one behind the other. The forward (cephalic) one is derived from the 2nd pouch and is known as the thymus II; the hinder (caudal) from the 3rd pouch is then the thymus III. These are constantly present, and in the Lacertilia, constantly separated from one another (Figs. 2, 3). They extend, on either side, from the carotid arch forwards (cephalad) towards the base of the skull, in company with the vagus nerve, the internal carotid artery and the internal jugular vein. Near the base of the skull, the thymus II comes to lie posteriorly rather than laterally to the pharyngeal wall, owing to the fact that, at this level, as stated by

Appleton (1929), the pharynx itself projects laterally, as the recessus piriformis, towards the tympanic cavity (see Wyeth, 1924, Fig. 18). At its cephalic extremity the thymus II is crossed superficially by the hypoglossal nerve which curves forwards and ventrally towards the hyoid region. Variations in the shape of these two lobes are common, and, as already indicated, we may also find other masses of thymus tissue situated more caudally (Fig. 5).

In *Lacerta* and in *Sphenodon*, the thymus II and III are two somewhat elongated, flattened structures, white in colour, overlapping one another slightly at their opposed extremities (Figs. 2, 3). I have been able to confirm van Bemmelen's statement that they receive their blood supply from the arteria muscularis cervicis which is usually the only branch of the ductus caroticus (Figs. 2, 3).

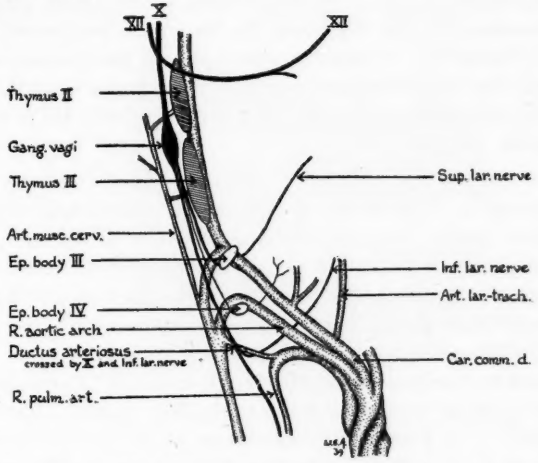

Fig. 3. *Sphenodon*. A semidiagrammatic view of the cervical region from the right lateral aspect.

It is now accepted that the lacertilian thymus undergoes an age involution. This has been described by Simon (1844) and Maurer (1899). It has also been discussed by Hammar (1911). Saint-Remy & Prenant (1903) show in their Fig. 13 an enormously large thymus in an embryo, relatively much larger than it ever appears in the adult. I have noticed this also in *Sphenodon*, where, in a new-born specimen, a very large thymus III extended so far caudally as to enclose completely the epithelial body III, whereas in the adult *Sphenodon*, it is always much smaller, and is rostral of the epithelial body, to which, however, it may be connected by a distinct strand of dense connective tissue (van Bemmelen). Maurer (1899) states, that even in late embryos of *Lacerta*, it may be connected to the epithelial body III by lymphoid tissue.

The detailed histological features of the lacertilian thymus are being investigated, and will be the subject of a separate communication.

THE EPITHELIAL BODIES III AND IV

No useful purpose will be served by discussing at length the various arguments concerning the significance of these structures. The ground has been well covered and the problem largely resolved by Maurer (1899), Kohn (1899) and Trinci (1912). More recently, Pischinger (1937) has again reviewed the subject fully. It is sufficient to say that it is now definitely established that these structures are epithelial in origin, being derived from the ventral parts of the pharyngeal pouches III and IV. They retain in the adult their embryological relationship to the definitive arteries formed from the corresponding vascular arches, viz. the carotid arch and the aortic (systemic) arch, a fact which has been responsible for the confusion which existed for a long time between these structures, and bodies of quite a different nature, which possess a corresponding relation to the same vascular regions in the Mammalia (see below). According to the usual description, *Sphenodon* alone, among the Sauria, retains both bodies on each side (see below), whereas *Lacerta* and other lizards, it is usually stated, present only one on each side—the epithelial body III in relation with the carotid arch.

Epithelial body III

This is present in *Lacerta* and in *Sphenodon*, as a faintly yellowish, discoidal structure, firmly attached to the wall of the carotid arch just at the point where it passes over into the ductus caroticus, and the internal carotid artery is given off (Figs. 2–4). The body itself is barely visible in *Lacerta*, but is quite obvious in *Sphenodon*, where it is surrounded by an accumulation of pigment cells which give to it a dense black appearance.

It was called by van Bemmelen, who first described it, "Carotiskörperchen", and has since been given other equally misleading names ("Carotid Body"; "Carotid Gland"). As Trinci (1912) has shown, all these names unfortunately suggest an homology with the structure which bears similar names in the Mammalia, whereas, due largely to his paper, it is now well known that it has nothing at all in common with the carotid body (glomus caroticum) of mammals (see also Maurer, 1899; Weber, 1909). On the contrary, both it and the epithelial body IV have a genetical correspondence with the mammalian parathyroids. Whether, however, they are functionally homologous with the latter we do not know, and experimental evidence on this important point is desirable. Shapiro (1933) has indeed investigated the same structures in the amphibian *Xenopus* with this aim in view; his description of their histology, however, is not entirely satisfactory. (See also Waggener, 1930.)

In spite of the conclusive evidence as to the morphological significance of the epithelial body III, evidence which has been accepted by all authorities (see Pischinger, 1937), its nature continues to be misinterpreted. Bhatia & Dyal (1933) have figured the epithelial body III in the lizard *Hemidactylus* as if it were interposed upon the common carotid, in a manner similar to the "carotid gland" of the Amphibia. Ask-Upmark (1935), in a comprehensive

investigation into the morphological basis of reflexes from the carotid sinus and glomus caroticum, examined a number of reptiles, but, as he freely admits, was unfortunately not able to examine a lacertilian. He cannot be excused on this account, however, for misinterpreting the epithelial body III as the homologue in reptiles, of the mammalian glomus caroticum. In his figures of certain ophidians, the "carotid body" to which he refers is none other than the epithelial body III, and presumably, therefore, cannot play the part in these vascular reflexes which he ascribes to it. For the Lacertilia, while he rightly criticizes the figure of Bhatia & Dyal, he is nevertheless mistaken in regarding the structure which they call "carotid body" as the homologue of the mammalian carotid body, and as having the same physiological significance.

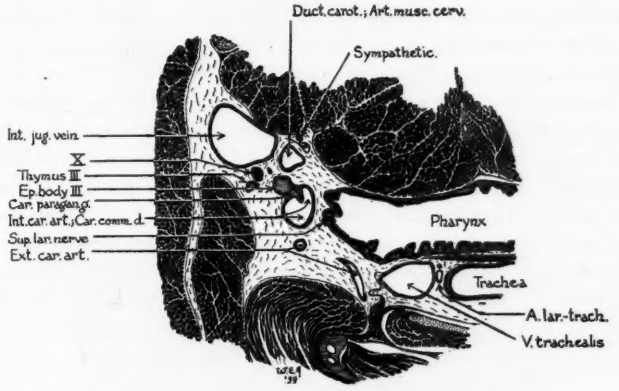

Fig. 4. *Lacerta*. Transverse section of the right side of the neck, approximately at the level A in Fig. 2.

Epithelial body IV

According to all accounts this is absent in all the Sauria except *Sphenodon*. Wettstein (1931), in fact, expressly cites this as one of the distinguishing features of *Sphenodon* (p. 3). I feel that this is not necessarily the case, since, in an adult *Lacerta*, I have seen an epithelial body IV on the left side, embedded in a mass of thymus tissue (Fig. 5). Verdun (1898) also records the occurrence of the epithelial body IV in the Lacertilia. Indeed, in *Lacerta* these caudal epithelial bodies are usually seen to appear early in development, and to disappear again before birth; their occasional persistence, therefore, is not at all surprising, and closer observation may show that it is not unusual. In *Sphenodon* (Fig. 3), in which the epithelial body IV invariably persists, it has the same relation to the aortic (systemic) arch as the epithelial body III has to the carotid arch. Van Bemmelen was the first to discover it in *Sphenodon*, and called it the "Aortakörperchen". In size and shape it is very similar to the epithelial body III, but it lacks the dense surrounding pigmentation of the

latter. O'Donoghue (1920) states (p. 193) that van Bemmelen gave the name "oesophageal gland" to a "slight swelling" of the wall of the systemic arch in *Sphenodon*. I can find no reference to such a statement by van Bemmelen, either in the work cited or in any other of his papers. In the account to which O'Donoghue specifically refers, van Bemmelen (1888) states quite definitely that the body in question ("Aortakörperchen") resembles the "Carotiskörperchen" and bears a corresponding relation to the aortic arch.

Histologically, the epithelial bodies are compact organs presenting a somewhat lobulated appearance, and surrounded by a definite connective tissue capsule which, in *Lacerta*, is only slightly pigmented. As mentioned above, Thompson (1911) makes the interesting point that the epithelial cells sometimes surround lumina and may even line vesicles. This fact, however, had been observed previously by Weber (1909) in the Lacertilia. It had also been noticed in the mammalian parathyroid by Kohn (1899), Alagna (1908) and others. It is referred to by Biedl (1916) and Schafer (1929), both of whom state that the lumen of the vesicles may become distended with a "colloid", which, according to Schafer, does not contain iodine. The interest of this lies in the fact that it may account for the confusion of a "vesicular" epithelial body ("parathyroid") with other epithelial structures which are normally of a vesicular nature (thyroid and ultimobranchial body). In sections of the epithelial body of *Lacerta* I was not able to observe an arrangement of the epithelial cells into vesicles, nor the presence of lumina such as Thompson and Weber described. Between the cords of epithelial cells, however, numerous capillaries can be observed, and the whole gland is very vascular.

THE ULTIMOBRANCHIAL BODY
(Greil, 1905)

This structure is found in all vertebrates (except cyclostomes and teleostean fishes) at some stage in development. In the different classes of vertebrates it has received several different names—"suprapericardial body" by van Bemmelen who discovered it, "accessory thyroid" by de Meuron, "postbranchial body" by Maurer, and "lateral thyroid" by Born (1883) and others, who believed that it enters into the formation of the lateral part of the mammalian thyroid gland. Although it is generally agreed that the lacertilian ultimobranchial body is homologous with the structure bearing the same name in the mammals, yet in the Lacertilia it has no connexion whatsoever with the thyroid gland. As the condition in *Lacerta* therefore has no real bearing on the problem as it exists in the Mammalia (see Kingsbury, 1935) there is no reason to discuss the numerous interpretations as to its significance, which relate principally to the conditions in the mammals.

In *Lacerta*, the ultimobranchial body can be identified as an independent structure, persisting, in the adult, only on the left side. It is situated laterally to the trachea, and adjacent to the arteria laryngo-trachealis and the inferior

laryngeal nerve (Figs. 5, 6); it is caudal of the thyroid gland and dorsomedial of the left common carotid artery. It has no close relationship with the

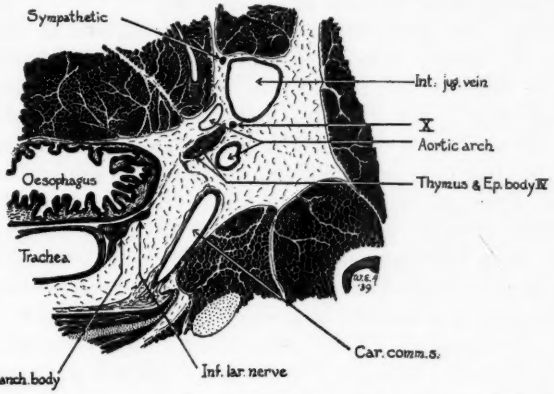

Fig. 5. *Lacerta.* Transverse section of the left side of the neck, approximately at level B in Fig. 2.

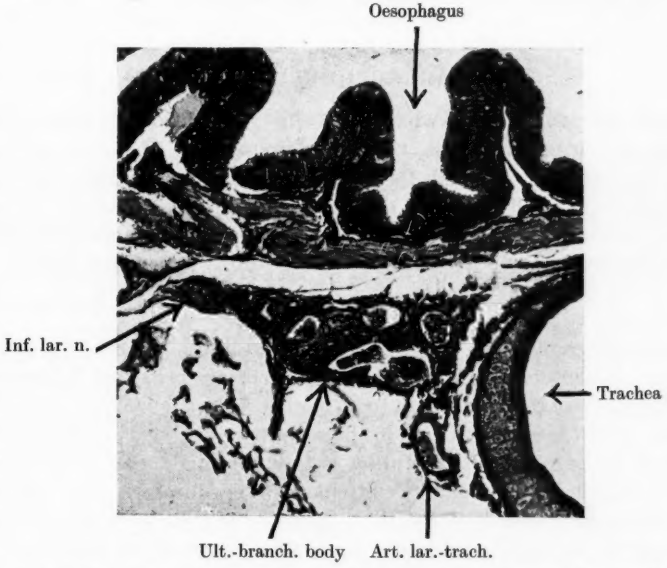

Fig. 6. *Lacerta.* Low-power photomicrograph of left latero-tracheal region showing the ultimobranchial body. The section is about the level of Fig. 5, but is reversed.

"parathyroid gland" (epithelial body III) as maintained by Thompson (1911). I can uphold van Bemmelen's criticism of de Meuron's (1886) figures, which show the ultimobranchial body in a position ventral to the left common

carotid, whereas, as stated, it is actually just dorsomedial of this. He also over-exaggerates the size of the ultimobranchial body since it would scarcely be visible in a figure of the scale of his figure 15.

According to Maurer (1899) it is formed of a number of vesicles lined by epithelial cells, usually of a simple cuboidal type. One of these vesicles is much larger than the others and may represent the original diverticulum ("Mutterbläschen"); this, he states, contains colloid while the smaller vesicles which surround it have a very small lumen and are apparently empty. Krause (1922) on the other hand says that none of the vesicles contains colloid. I have found that, although there are many small vesicles apparently empty, yet several vesicles of larger calibre can be identified, all of which appear to enclose a material, often homogeneous but occasionally containing cells which seem to be desquamated epithelial cells. The epithelium lining the larger vesicles is usually formed of low cuboidal cells which may be so flattened in places as to be difficult of recognition. The vesicles are bound together by dense connective tissue, thus producing an organ which is fairly compact and somewhat conical in shape; it is, however, microscopic in size (Fig. 6).

The ultimobranchial body has not yet been located in *Sphenodon*, in which it is said to be absent (van Bemmelen, 1889; Wettstein, 1932).

"PARAGANGLIONIC" TISSUE

Certain specialized cells which were first identified by Trinci (1912) especially in the regions of the truncus arteriosus and of the carotid arch, deserve mention here, although they are undoubtedly not of branchial origin. These cells lie, generally diffusely distributed, in the adventitia of the vessels concerned, and therefore do not appear as compact and discrete structures. Taken collectively, Trinci differentiated them into two principal groups.

Cardiac "paraganglion"

This embraces all those cells which are found in the region of the truncus arteriosus. It is interesting to note that Trinci long ago predicted the discovery in the Mammalia of cells equivalent to his cardiac paraganglion; such specialized cells have indeed been since found in the corresponding region in Mammalia—cells which enter into the constitution of the paraganglion aorticum and the glomus aorticum of mammals (Penitschka, 1931; Muratori, 1932; Nonidez, 1936). Although Trinci favoured the association of these cells with the chromaffin system, their real significance is as yet unknown in the Reptilia.

Carotid "paraganglion"

This group of specialized cells is found in relation with the carotid arch at the origin of the internal carotid artery and adjacent to, but having no connexion with, the epithelial body III. On morphological grounds, Trinci considered these cells to correspond to those of the mammalian glomus

caroticum. They are quite distinguishable in *Lacerta* (Fig. 4), where they lie in the adventitia of the carotid arch at the point of origin of the internal carotid artery. Numerous pigment cells are in relation, and I have also observed very fine branches of the superior laryngeal nerve passing to this important region. Whether or not these nerves have any connexion with the cells in question has not yet been ascertained. Trinci believed that the cells which he had discovered, like those of the carotid "paraganglion", formed part of the extracapsular chromaffin system, but in spite of the presence of chromaffin cells, is it not possible that they may be on the afferent side, and thus represent cells homologous with those of the mammalian glomus caroticum? It is proposed to investigate further both this possibility, and another—that they may prove to be non-chromaffin paraganglia of the vagal type (Goormaghtigh, 1936).

It is, of course, these particular cells which might easily have provided Ask-Upmark with a suitable morphological basis for his study of the vascular reflexes, had he known of them and investigated their function. The mere identification of such cells, indeed, proves nothing as to their function. Trinci has laid an excellent foundation, but extensive investigations are still required concerning the development of these cells and their relationship with the nervous system, together with some form of experimental approach. Until this has been accomplished it will be impossible to confirm or deny Trinci's assumptions as to their function, assumptions which were based purely on their chromaffinity.

SUMMARY

1. The thyroid gland of *Lacerta* is described and figured. It presents no obvious histological differences from that in other vertebrates. It has also been confirmed that it has a double blood supply, from the external carotid artery and from the arteria laryngo-trachealis, which is a branch of the pulmonary artery. Certain statements in a recent paper, relating to the thyroid of *Egernia*, are questioned.

2. The thymus gland of *Lacerta* and *Sphenodon* consists of two "lobes" derived from the second and third pharyngeal pouches, and known as thymus II and III. They lie one behind the other, adjacent to the internal carotid artery, internal jugular vein and the vagus nerve. The fact, mentioned by van Bemmelen for *Sphenodon*, that they are supplied by the arteria muscularis cervicis, is now confirmed for *Lacerta*. An age involution of the thymus has also been observed in *Sphenodon*.

3. In *Sphenodon* there are two epithelial bodies, known respectively as III and IV. The epithelial body III is attached to the wall of the carotid arch, adjacent to the origin of the internal carotid artery, while the epithelial body IV occupies a corresponding relation to the aortic (systemic) arch. In *Lacerta*, it is generally assumed that there is usually only one epithelial body on each side —the epithelial body III. Mention is made, however, of the occurrence of a small epithelial body IV, embedded in thymus tissue, with which it lies in the

concavity of the aortic arch. Histologically these structures consist of a compact mass of epithelial cells, which were not observed to surround lumina or vesicles.

4. The ultimobranchial body is constantly present on the left side in *Lacerta*, lying on the lateral aspect of the trachea, just behind the left common carotid artery. It is of microscopic dimensions, and consists of a number of epithelial vesicles, of varying size, and bound together by connective tissue.

5. Specialized cells have been identified in *Lacerta*, situated within the adventitia of the carotid arch, at the point of origin of the internal carotid artery, and at the same level as the epithelial body III. They have no connexion with the epithelial body, and obviously correspond to the "carotid paraganglion" which Trinci has described. Certain minute branches of the superior laryngeal nerve were seen passing to the arterial wall in this vicinity, and the significance of this is briefly discussed.

I wish to express my thanks to Prof. W. P. Gowland, University of Otago, New Zealand, where this work was commenced, both for allowing me the opportunity of dissecting some specimens of *Sphenodon* and for his stimulating interest in the earlier stages. I am indebted further to Prof. Durward, of the Department of Anatomy, Leeds University, for facilitating the subsequent progress of the investigation, and for his encouragement and advice at all stages. The sections were prepared by Mr A. W. Dent, for whose assistance I am also grateful.

ABBREVIATIONS

Art. (A.) lar.-trach.	Arteria laryngo-trachealis.
Art. musc. cerv.	Arteria muscularis cervicis.
C. branch. II	Cornu branchiale II.
Car. comm. s. and d.	Left and right common carotid artery.
Carot. interna	Internal carotid artery.
Car. paragang.	Carotid "paraganglion".
Comm. pul. tr.	Common pulmonary trunk.
Dors. aorta	Dorsal aorta.
Duct. carot.	Ductus caroticus.
Ep. bodies III and IV	Epithelial bodies III and IV.
Ext. car. art.	External carotid artery.
Gang. vagi	Ganglion nodosum of the vagus.
Inf. lar. nerve	Inferior laryngeal branch of vagus.
Inf. thyr. art.	Inferior thyroid artery
Int. car. art.	Internal carotid artery
Int. jug. vein	Internal jugular vein.
L. syst. trunk	Left systemic (aortic) trunk.
L. pulm. art.	Left pulmonary artery.
R. pulm. art.	Right pulmonary artery.
R. syst. arch	Right systemic (aortic) arch.
Sup. lar. nerve	Superior laryngeal branch of vagus.
Sup. thyr. art.	Superior thyroid artery.
Ult.-branch. body	Ultimobranchial body.
V. trachealis	Vena trachealis.
IX	Glossopharyngeal nerve.
X	Vagus nerve.
XII	Hypoglossal nerve.

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THE OCCURRENCE OF THE LEMURINE FORM OF THE ECTOTYMPANIC IN A PRIMITIVE MARSUPIAL

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The peculiar condition of the tympanic annulus (os ectotympanicum) in relation to the auditory bulla in the lemurine lemurs is well known. In all the Lemuriforms, the annulus tympanicus is overgrown by, and ensnared within, the petrous inflation that forms the bulla. This peculiarity is shared by the Tupaioidea and constitutes one of the many bonds of affinity that unite the tree shrews with the lemurs. It seems to have been assumed by many writers upon the phylogeny of the Primates that this ensnaring of the annulus tympanicus is a unique phenomenon in the tree-shrew-lemur phylum, no other mammalian group sharing in this peculiarity. It is therefore of interest to learn that it occurs in exactly the same basal condition in an altogether different group of mammals.

In the course of a comprehensive study of the pneumatization of the skull in the region of the tympanic cavity, the auditory bulla of *Dasycercus cristicauda*[1] has been examined both in the "pouch" young and in adult stages. *Dasycercus* is a primitive, practically pouchless, didactylous, polyprotodont marsupial. In many ways it is one of the most generalized of the existing Australian Didelphia, and attention has been drawn in previous publications (Wood Jones, 1923*a*, *b*) to some of the strangely primitive, almost reptilian, attributes of this small insectivorous marsupial. In this animal the degree of bulla inflation is excessive and, in the growth of the bulla, exactly the same result has been attained as that seen in the tree-shrew-lemur assemblage, for the tympanic annulus has been completely overgrown by the bulla and has been left enveloped within the bulla cavity. There are, however, some interesting differences in detail between the enclosed ectotympanic of *Dasycercus* and that of the tree-shrew-lemur phylum.

In the tree shrews, the bulla is formed by an inflation of an entotympanic element, with, in addition, a small tympanic extension of the alisphenoid which contributes towards the formation of the roof of the tympanic cavity. In the lemuriform Lemurs, this backward extension of the sphenoid just comes up to the bulla wall, but makes no contribution to its actual formation (see Fig. 1). In *Dasycercus*, the sphenoid inflation is responsible for the greater part of the bulla cavity, the petrous and exoccipital forming only its hinder portion (see Fig. 2). This is, of course, only an exaggeration of the condition which is

[1] *Dasycercus cristicauda* Peters, 1875; *Chaetocercus cristicauda* Krefft, 1866 (preoccupied); *Phascologale cristicaudata* Thomas, 1887; *Amperta cristicauda* Cabrera, 1919.

characteristic of the Didelphia in which the sphenoid typically enters into the formation of the bulla. It is a fact of the greatest significance that the typical Didelphian sphenoid contribution to the formation of the bulla should be found again in *Ptilocercus* and in diminishing degree in *Tupaia* and Lemur. The fate of the encapsuled annulus tympanicus appears to differ somewhat in the two cases, for in *Dasycercus*, the imprisoned annulus develops an osseous tympanic external auditory meatus, which grows to the bulla mouth, and so forms a bony meatus, within the limits of the bulla inflation (see Fig. 3). The membrana tympani is situated deep within the cavity of the bulla, as in the

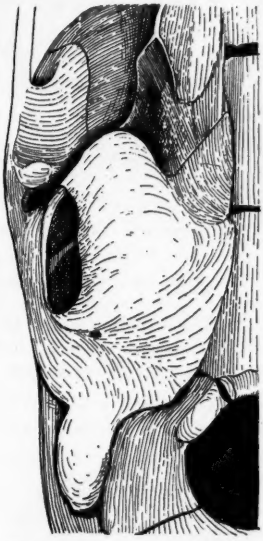

Fig. 1. The right bulla of a lemurine lemur. The sphenoid ends at the anterior aspect of the bulla.

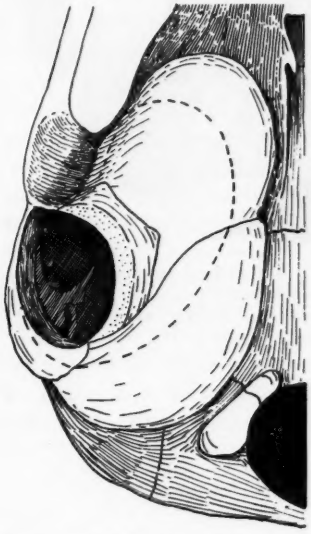

Fig. 2. The right bulla of a suckling *Dasycercus cristicauda*. The sphenoid forms the major portion of the bulla. The dotted line indicates the portion removed in Fig. 4.

lemurs: but it differs from that of the lemurs in that it is at the bottom of a bony tube, an imprisoned external auditory meatus, which leads to the surface of the skull at the bulla mouth (see Fig. 4).

In the dried lemur skull the annulus is left stranded in the midst of the bulla, attached to the bulla wall only by its upper edge (see Fig. 5). There is no bony meatus leading from the annulus to the bulla mouth. The condition therefore appears, at first sight, rather different from that typical of *Dasycercus*. But in the recent skull the likeness between the two animals is very exact, for the "intra-bullary" auditory meatus of the lemur, though not composed of bone, is an exact replica of the condition seen in *Dasycercus* (see Fig. 6). In *Dasycercus*, the fibrocartilaginous auditory meatus of the tree-

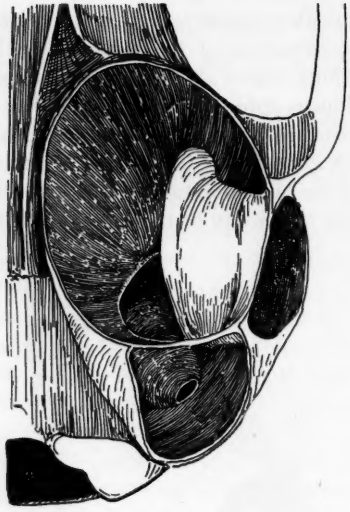

Fig. 3. Left bulla of an adult *Dasycercus cristicauda* opened to show the ectotympanic and bony auditory meatus.

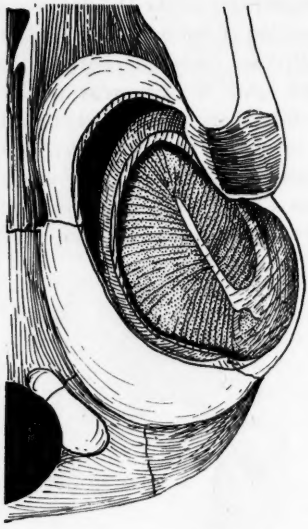

Fig. 4. Left bulla of a suckling *Dasycercus cristicauda* opened along the line shown in Fig. 2 to show the ectotympanic and membrana tympani after removal of the greater part of the bony auditory meatus.

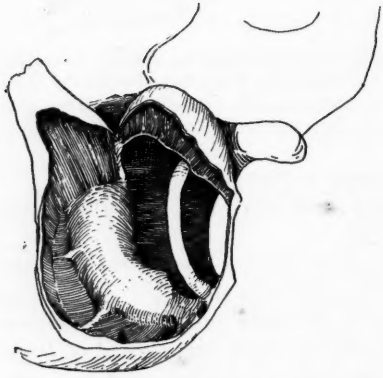

Fig. 5. Left bulla of a lemurine lemur opened to show the encapsuled ectotympanic. The skull has been macerated and the fibrocartilagenous auditory meatus is absent.

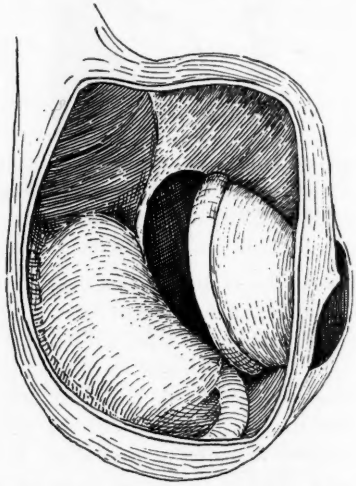

Fig. 6. Left bulla of a lemurine lemur opened in the recent state to show the fibrocartilagenous auditory meatus. Compare with Fig. 3.

shrew-lemur assemblage has been replaced by bone. It would be a matter of great interest if, in the examination of a large series of skulls of tree shrews, examples showing ossification, partial or complete, of the "intra-bullary" auditory meatus should be met with. Such a series is, unfortunately, not available to us.

It is likely to be more than a mere coincidence that such ancient and generalized Monodelphians as the tree-shrew-lemur phylum and such a generalized, pouchless, Didelphian as *Dasycercus* show the same remarkable features in the relation of the annulus tympanicus and bulla formation. It is a possibility that in *Dasycercus* and *Ptilocercus* we have living mammals that are situated near to the ancient meeting point of the Monodelphia and Didelphia.

In certain other marsupials (Dasyuridae and Peramelidae) in which the ectotympanic forms an external auditory meatus protruding from the bulla, the membrana tympani is sunk somewhat within the cavity of the bulla as was described and figured by Van Kampen. This author, however, did not examine *Dasycercus*, and Carlsson who described the bulla of *Dasyuroides*, which in many intimate features is obviously nearly allied with *Dasycercus*, dismissed the ectotympanic by saying: "The lateral part of the bulla is related to the tympanic ring, which forms part of the external auditory meatus as in the whole of this order of animals." The condition found in *Dasycercus* is peculiar in that the ectotympanic and its derived external auditory meatus are wholly within the bulla; in the forms described by Van Kampen the ectotympanic grows from the bulla mouth as a true tympanic external auditory meatus. In this regard, *Dasycercus* may be said to find its parallel with the lemurine, and certain Peramelidae and Dasyuridae (and to some degree the general run of the Didelphia) more with the lorisine lemurs.

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RADIOGRAPHIC STUDIES OF THE METACARPAL AND METATARSAL BONES IN CHILDREN

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INTRODUCTION

This paper is the first of a series devolving from the periodic radiographic examination of 200 normal primary school children. The study was inaugurated by the late Prof. H. H. Woollard in an attempt to determine the part played by nutrition in the maturation of bones, and to divorce this, if possible, from other factors. The present paper is concerned with the appearance of epiphysial irregularities such as true double or so-called "pseudo-epiphyses" in metacarpal and metatarsal bones, which, it was thought, might shed some light upon this problem, since an increase in the incidence of pseudo-epiphyses has been recorded in cases of retarded growth (Siegert, 1910; Rochlin, 1927).

True supernumerary epiphyses have a separate centre of ossification. Pseudo-epiphyses arise from a diaphysial extension into the cartilaginous extremity of the bone (Uffelmann, 1863). In the early stages of development a rod of osteoblastic tissue arising from the metaphysis invades the chrondro-epiphysis. The distal part of this rod enlarges, so that later the ossified shaft appears to be surmounted by a mushroom-shaped cap. The connecting stalk may be narrow, or it may be so thick that it almost completely supplants the intervening cartilaginous ring. Thus the radiographic appearances vary from well-marked marginal incisions to shallow indentations of the bony cortex (Pl. I, fig. 1). When the connecting stalk is thin, it is sometimes difficult to discriminate in X-ray pictures between a true and a pseudo-epiphysis. This is especially the case where the shapes of the adjacent bony margins lead to inevitable overlapping of the shadows, as frequently occurs in the first metatarsal. On the other hand, slight degrees of pseudo-epiphysial formation must be differentiated from linear brightenings associated with the development of articular facets which may occur at the extremities of the metacarpals.

METHOD

The hands and feet of children from 4 to 8 years were examined radiographically at intervals of 6 months, for a period of 18 months. For the purposes of the present study, radiographs of 100 cases were selected. General clinical and dental examinations, including measurements of height, weight and muscular power, were also carried out. A vitamin C saturation test was performed, in order to determine whether low storage values had any influence

on bone maturation. The children were given orally 100 mg. of ascorbic acid, and the amount of vitamin C excreted in the urine after 2–3 hr. was determined.

FINDINGS

Undoubted true double epiphyses were only seen in two cases, the supplementary epiphysis being in both at the distal end of the 1st metatarsal. Pseudo-epiphyses, on the other hand, were common. They were seen in 96 % of the children's hands.

Table I. *Percentage incidence of pseudo-epiphyses in hands and feet of children*

	1st	2nd	3rd	4th	5th
Metacarpals	81	52	1	2	57
Metatarsals	57	0	0	0	1

The irregularities recorded were nearly always bilateral. In two cases pseudo-epiphyses were observed on one side only. It has been stated (Stettner, 1931) that the radiographic markings of pseudo-epiphyses can persist for many years. In a number of cases the metacarpal pseudo-epiphyses had undergone considerable changes even in the comparatively short period of 18 months, some of them becoming more, some less marked, and others disappearing completely.

Table II. *Changes in the percentage incidence of pseudo-epiphyses after eighteen months*

	Appear more marked	Appear less marked	No longer evident
1st metacarpal	5	—	5
2nd ,,	17	—	12
3rd ,,		No change	
4th ,,		No change	
5th ,,	—	8	—

Comparison between the radiographic findings and the height, weight, muscular power and dental examinations showed no correlation between pseudo-epiphysial formation and the general nutritional state of the children as judged by these standards. In two children with mild degrees of Frölich's syndrome there were no pseudo-epiphyses. In seven children with slight clinical signs of rickets (bowed tibiae and enlarged costochondral junctions) there was no increased incidence. This is in agreement with other observers (Josefson, 1916; Hasselwander, 1921; Koehler, 1935). In almost all the children a remarkably low level of vitamin C storage was found. This was also unrelated to the nutritional status as exemplified both by the clinical findings and the radiographic appearances.

DISCUSSION

The percentages in Table I are in accordance with previous statistics. Stettner (1931), who published figures from 538 cases, found approximately the same percentage of pseudo-epiphyses and a similar distribution. The preponderance of the 1st metacarpal and metatarsal is striking. The 2nd and 5th metacarpals figure next with nearly equal frequency, while the 3rd and 4th metacarpals seldom show pseudo-epiphysial formation. The 2nd–5th metatarsals remain almost entirely free. The distribution seems to follow a definite pattern, for which up to now no satisfactory explanation has been offered. Possibly the appearance of these irregularities could be correlated with the relatively extensive mobility of the 1st, 2nd and 5th fingers and the great toe. Uffelmann believed that the 1st metacarpal represents a combination of a phalanx and a metacarpal, and suggested that this might explain the frequency of pseudo-epiphyses in this bone. However, this would not explain the appearance of pseudo-epiphyses in the other bones.

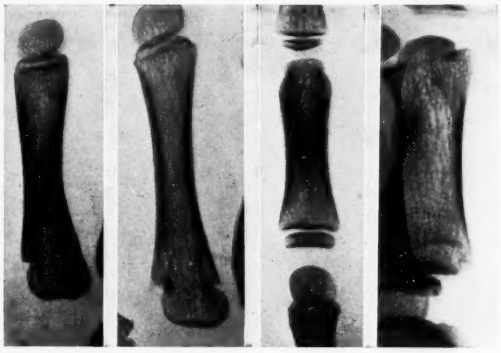
Stettner observed in another series that, while on excluding the 1st metacarpal one-third of all "normal" children showed pseudo-epiphysial formation, two-thirds of all those "congenitally stigmatized" (e.g. with congenital dislocation of the hip), and about half the number of those with "exogenetic" diseases, showed the condition.

From these findings the author concluded that any early damage to the embryo may be reflected later in the formation of a large number of pseudo-epiphyses. That he should have excluded the 1st metacarpal from his statistics diminishes the significance of his results. Notwithstanding the absence of definitely labelled pathological material in our series and the small numbers examined, from our observations we see no reason to associate an occurrence so common as pseudo-epiphysial formation with congenital or endocrine disturbances.

Koehler (1912) has suggested that the occurrence of either true or pseudo-epiphyses in man might be due to the appearance of osteogenetic characters suppressed during evolution, and points to the well-known fact that in slowly growing sea-mammals (seal, walrus and whale) true double metacarpal epiphyses occur regularly. Monteiro & Carvalho (1938) have described a case in which they found radiographically a complete set of true double epiphyses in a healthy child. Thus, since neither nutritional factors nor pathological processes appear to influence the appearance of pseudo-epiphyses, a true genetic mechanism may be postulated. This hypothesis, which can only be solved by familial research on a large scale, has not so far been put to the test.

SUMMARY AND CONCLUSIONS

1. A statistical analysis of the number of pseudo-epiphyses appearing in the metacarpal and metatarsal bones of one hundred children between the ages of 4 and 8 years has been made.



A B C D

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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2. No correlation could be found between the incidence of pseudo-epiphyses in metacarpal and metatarsal bones and the nutritional status of children as determined by height, weight, amount of dental caries and muscular power.

3. Pseudo-epiphysial formation does not appear to be correlated with vitamin deficiency or endocrine disturbances.

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EXPLANATION OF PLATE I

Fig. 1. Pseudo-epiphyses in A, base of 2nd metacarpal; B, base of 2nd metacarpal; C, distal end 2nd phalanx ring finger; D, distal end 1st metatarsal.

Fig. 2. The pseudo-epiphysis at the base of the 2nd metacarpal is attached to the shaft by a thin stalk.

Fig. 3. Pseudo-epiphyses at the bases of the 2nd and 5th metacarpals and distal end of 1st metacarpal.

Fig. 4. True supernumerary epiphysis at the distal end of the 1st metatarsal.

THE STRUCTURE OF THE EPIPHYSES IN *SPHENODON* AND THE PRIMITIVE FORM OF SECONDARY CENTRE

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INTRODUCTION

The epiphyses of the early tetrapods are now known to have consisted of pads of hyaline cartilage overlying the convex ends of the long bones, thicker in the younger and thinner in the older animal, but with no secondary centres of ossification or calcification (Haines, 1938). But it has long been known that secondary centres are found in *Sphenodon* and in lizards, and in several of the fossil representatives of the groups to which they belong, and also in some fishes, anurans, birds and mammals. The detailed structure of these centres has however been studied little, and it will be the purpose of this paper to describe their microscopic structure in *Sphenodon*, the most primitive animal possessing well-developed secondary centres, and then to discuss their evolution and their further phylogenetic development in some other groups of animals.

My thanks are due to the Thomas Smythe Hughes fund for a grant towards the purchase of material.

THE EPIPHYSES IN *SPHENODON*

Secondary centres in the spines of the vertebrae of *Sphenodon* were reported by Albrecht (1883), the earliest record of such centres in reptiles. Howes & Swinnerton (1901, Pl. 6, figs. 14, 18) figured centres of the distal ends of the radius and ulna, but did not describe them in the text. Moodie (1908) failed to find any trace of the centres in his cleared preparations, and doubted the accuracy of the earlier observations. His material however was fully adult, and his conclusion that epiphysial centres were rarely present in *Sphenodon* was unjustified. Lubosch (1910) appears to be the only author who has prepared or figured microscopic sections of the epiphyses, but though his figure of the metatarsal shows a large secondary centre, it is not clear enough to show whether it is made of bone or cartilage. Heidsieck (1929), who has given an excellent account of the microscopic structure of the long bones of *Sphenodon*, and who in his work on lizards (1928) has examined reptilian epiphyses, had no young material available for study, and states specifically (in his paper) that he was unable to determine the structure of the secondary centres.

Several young individuals are now available for study. A dried forelimb and hindlimb skeleton in the British Museum (Index Collection), a cleared tarsus in the Dendy collection at King's College, and a radiograph of a com-

plete individual at University College leave no doubt as to the existence of secondary centres, the last confirming Albrecht's observations of separate centres for the vertebral spines. Further, I have been able to prepare sections of bones from four individuals, of 105 mm. total length (about 55 mm. snout-vent length) given me by the late Dr McMaster, of 149 mm. snout-vent length by courtesy of the Zoological Society of London and of two subadults, one sent by Prof. Gowland and one of 225 mm. from Prof. Appleton's collection.

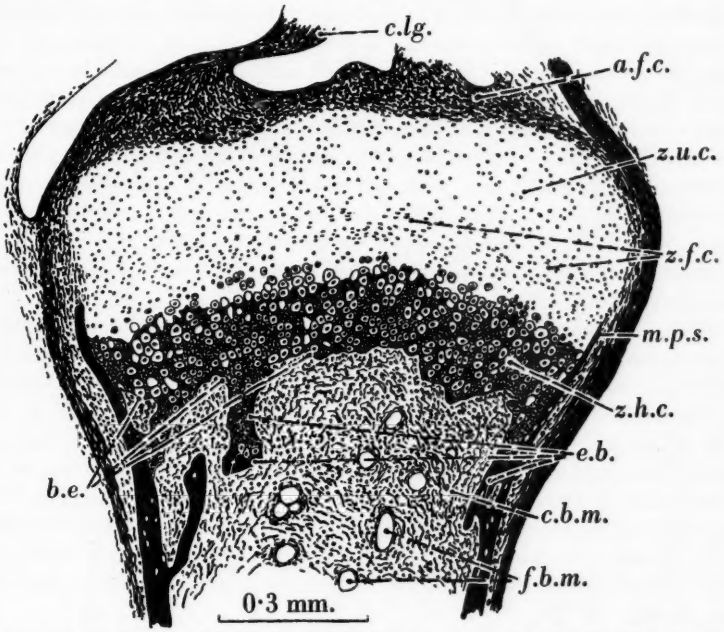

Fig. 1. Longitudinal section of the upper end of the tibia in a young *Sphenodon*. Total length 105 mm.

In the youngest specimen (serial of knee prepared), the essential epiphysial structure is well shown in the tibia but no secondary centres are yet developed. A thick articular fibro-cartilage (*a.f.c.*) covers the articular and non-articular surface, and gives attachment to the cruciate ligaments (*c.lg.*). The main mass of hyaline cartilage contains undifferentiated cells only (*z.u.c.*). At the level of the growing margin of the periosteal shaft (*m.p.s.*) this zone passes into the growth cartilage, a layer of flattened cells (*z.f.c.*) which is convexly bulged towards the articular surface. The zone of hypertrophied cells (*z.h.c.*) is well developed, and the calcification of the matrix is continuous, but the cells are as yet quite irregular in their arrangement. The surface facing the marrow is irregular, with large bays of erosion (*b.e.*) cut into the cartilage, indicating active destruction. Some endochondral bone (*e.b.*) has been laid down on the

cartilage, but it is not yet well developed. The marrow is cellular (*c.b.m.*), with a few scattered fat spaces (*f.b.m.*).

In the 149 mm. specimen (upper end of femur cut) the structure is profoundly altered by the formation of a secondary centre consisting of a mass of hypertrophied cartilage with a calcified matrix within the zone of undifferentiated cells. The endochondral bone is more fully developed. Since however the structure is similar to that of the next individual in the series, which is much better preserved, it will not be described in detail.

In Prof. Gowland's animal (all long bones radiographed, serials of lower end of humerus, upper ends of tibia and fibula, and both ends of radius and ulna prepared) well developed centres of calcified cartilage are found. In the lower end of the ulna for example (Figs. 2, 4) the whole cartilaginous epiphysis is massive, with a thin layer of fibro-cartilage on the articular surface (*a.f.c.*). Towards the centre of the cartilage the undifferentiated cells (*z.u.c.*) become hypertrophied (*h.c.s.c.*) and scattered calcifications of the matrix appear amongst them (*c.c.m.*). Finally the calcifications run together to form a continuous mass (*c.c.s.c.*). The secondary centre must be taken to include all the region of hypertrophied cells and calcified matrix whether the calcification is continuous or not.

Within the centre are numerous spaces (*s.s.c.*) of peculiar structure, hatched in the figures. Each is completely surrounded by calcified matrix, so that the cavities are never in direct contact with hyaline cartilage. The walls are very irregular, with many fragments of matrix apparently free in the cavities (Fig. 2, *i.f.c.m.*). It seems most probable that the cavities are artifacts, produced by the solution of calcium salts in places where the non-calcareous matter is insufficient to retain its continuity after decalcification. Certainly in radiographs of the epiphyses before calcification there is no sign of cavity formation in the secondary centres. Similar cavities have been seen in certain lizards, and they have been figured by Erdheim (1914) in the heavily calcified costal cartilages of rats.

The cells of the growth zone (Fig. 4, *z.g.*) are less flattened than before, and are arranged in clumps, while the hypertrophied cells form more or less definite longitudinal columns (*c.cl.*). The endochondral bone is plentiful and it now plays an important part in maintaining the strength of the formation, for the periosteal bone has remained relatively thin (Fig. 2, *p.b.s.*). It is arranged predominantly as longitudinal trabeculae (*l.t.*). The other epiphyses of the humerus, radius and ulna have a similar structure, each having one large calcified centre. Even at the lower end of the humerus, where the centre stretches from medial to lateral epicondyle, it is not subdivided. In the upper end of the radius, however (Fig. 3), there is no centre, probably on account of the relative thinness of the cartilaginous epiphysis, but the arrangement of the endochondral bone is similar to that described in the ulna.

In the fully developed bone the distinction between the shaft and epiphysial region is lost (Figs. 5, 6). The whole of the growth cartilage and the

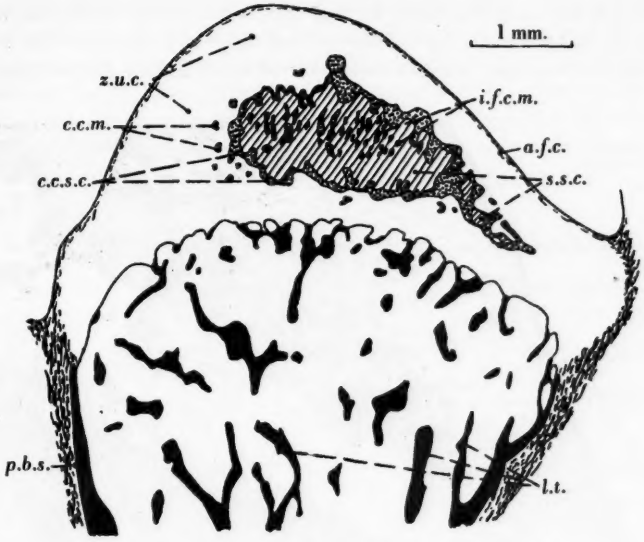

Fig. 2. Lower end of the ulna of a subadult *Sphenodon*.

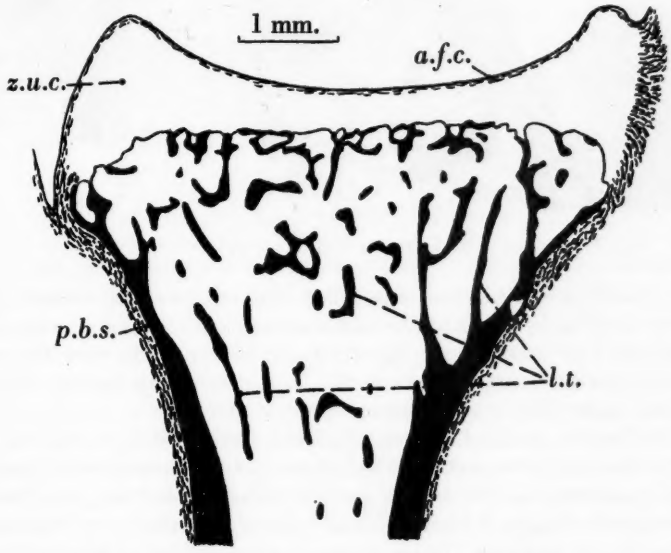

Fig. 3. Upper end of the radius of a subadult *Sphenodon*.

greater part of the calcified cartilage of the secondary centre are replaced by marrow and endochondral bone, so that the tissues of the shaft pass without interruption into the epiphysial region, and approach the articular surface. The remaining cartilage forms a thin layer of hyaline articular cartilage (Fig.

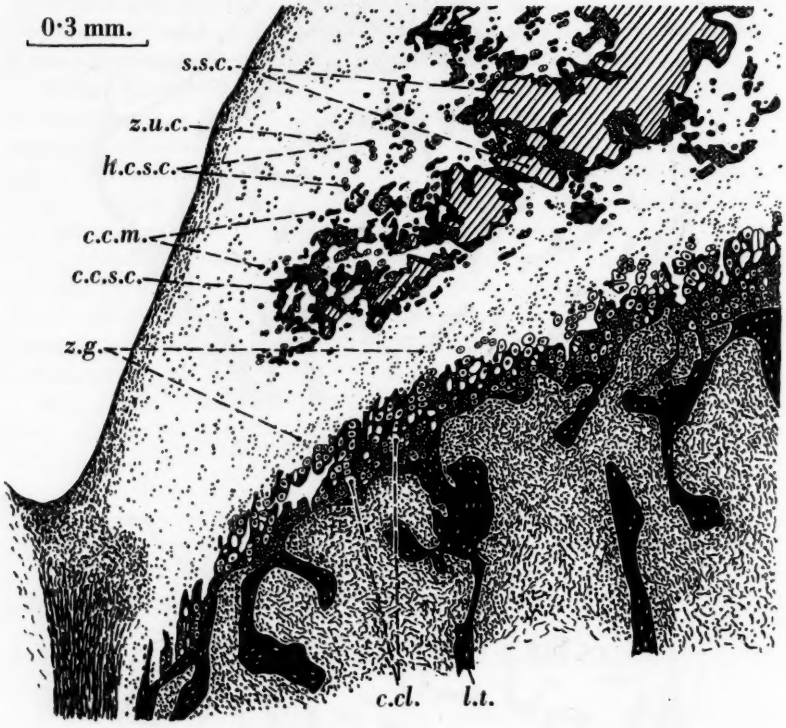

Fig. 4. Part of the lower end of the ulna. Same series as Fig. 2.

6, *a.c.*), beneath which a few unmodified cells represent the remains of the formerly wide zone of undifferentiated cells (*z.u.c.*). Passing towards the marrow the cells first become hypertrophied (*h.c.s.c.*), and then the matrix becomes heavily calcified (*c.c.s.c.*), this formation being derived from the uneroded subarticular part of the secondary centre.

In the shaft a cement line (*cem.*) separates the endosteal part of the endochondral bone (*e.e.b.*) from the periosteal bone (*p.b.s.*). The periosteal bone itself has now grown beyond the original limits of the shaft, and has come into contact with the cartilage of the epiphysial region (*con.*), the bone being sharply deflected by the cartilage. In the region of contact the cartilage has become calcified (*c.c.p.b.*).

The earlier epiphysial mechanism of growth is now destroyed, but it is possible that the bone is still capable of some slow growth. Some new cartilage may be formed by the division of the undifferentiated cells, and the bays of erosion (*b.e.*) have the appearance of activity. Again, though the marrow is for the most part fatty (*f.b.m.*), there is a thick layer of cellular marrow in contact with the cartilage (*c.b.m.*), not walled off from the cartilage by bone as is usually the case when erosion has ceased (Wallis, 1927).

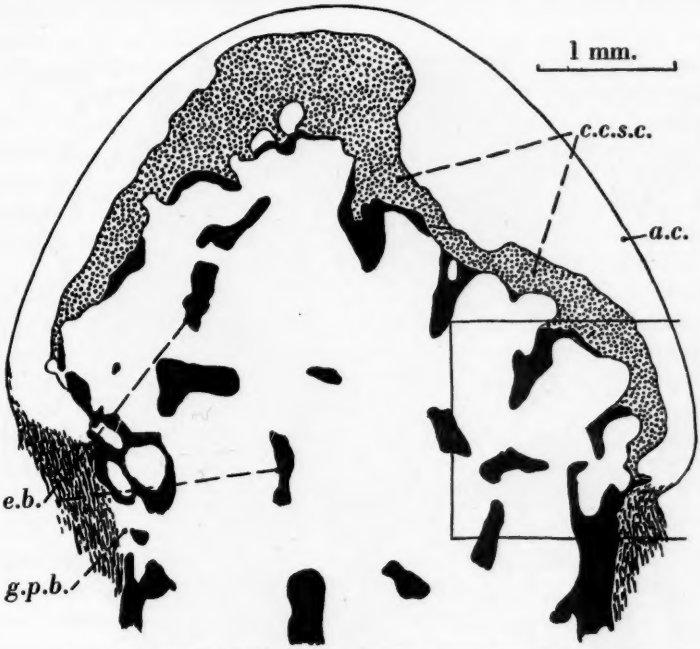

Fig. 5. Lower end of the femur of *Sphenodon*. Snout-vent ength 225 mm.

The tibia and fibula of Prof. Gowland's animal are similar in structure to the femur described. No specimen showing the actual stages of the destruction of the growth cartilage is known, so that the method of destruction must be inferred from the fully adult form. There are two possibilities. The secondary centre may be eroded independently by its own system of marrow and blood vessels derived directly from the perichondrium around the articular region, with the later secondary destruction of the growth cartilage, the method found in lizards and mammals. On the other hand the marrow of the shaft may first perforate the growth cartilage and then erode the secondary centre, so that the secondary centre never has at any time a blood supply independent of the shaft. Now even after the growth cartilage has been destroyed the original relationships of the vascular channels as they enter the bone are preserved,

and it is usually possible to determine whether any particular set of vessels originally entered the shaft or the secondary centre. In *Sphenodon* no vessel entering the epiphysial region has been seen in any bone, the most distal points of entry being definitely in the shaft (Fig. 5, *g.p.b.*), not through the cartilage of the epiphysial region as in lizards and mammals. In some bones the vessels are confined to the more central part of the shaft. So it seems that in *Sphenodon* the secondary centres are destroyed by the tissues of the shaft which perforate the growth cartilage on their way to the secondary centre.

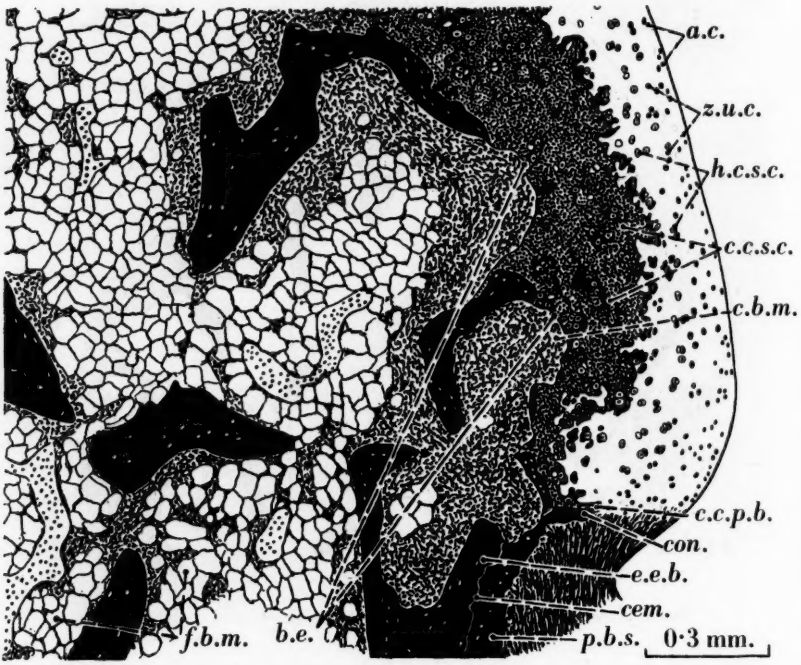

Fig. 6. Part of the lower end of the femur shown in Fig. 5.

Thus, if the appearances have been interpreted correctly, the epiphyses of *Sphenodon* pass through an early hyaline stage, this is followed by a prolonged period in which a large secondary centre of calcified cartilage occupies the region between the articular cartilage and the growth cartilage, and finally this is replaced by tissues derived in the shaft. Secondary centres have been recorded in the Jurassic *Sapheosaurus*, a fossil form related to *Sphenodon* (Meyer, 1859; Fuchs, 1908), the earliest record of such centres in any animal. So *Sphenodon* is probably typical of the Rhynchocephalia, and has probably preserved an early form of epiphysial structure.

THE PRIMITIVE FORM OF SECONDARY CENTRE

Large centres of calcified cartilage are known in anuran amphibians but are of a very specialized type, and smaller centres are also known in the branchial bones of some fishes (*Sciaena*, Haines (1934), and noted recently in the cod, *Gadus morrhua*). Similar centres which persist throughout life are known in some lizards (*Phyllodactylus*, Haines, unpublished work), but they are probably not primitive formations in this group. In all other animals in which such secondary centres are found at any stage (some birds, most lizards and mammals) the calcified cartilage is replaced by bone and marrow, derived directly from the perichondral tissues. But while in most eutherian mammals the calcified centre is small and transient, in monotremes (Haines, unpublished work) the centres are relatively large before they are eroded. It is thus reasonable to follow Parsons (1905) in suggesting that in the several distinct groups of animals in which secondary centres have been evolved, the first stage has in each case been the appearance of a small calcified centre in the depths of the cartilaginous epiphysis, as seen in the cod. The enlargement of this centre which still persisted throughout the life of the individual is a probable second stage, and *Sphenodon* in the persistence of the calcified centres to a late stage of growth appears so far primitive. The final destruction of the growth cartilage and replacement of the secondary centre by marrow and bone is probably a later evolutionary development, and though the structure in *Sphenodon* suggests that both processes were first carried out entirely by the tissues of the shaft, it must remain uncertain whether the lines leading to the lizards, birds and mammals have ever passed through such a stage in their phylogeny.

THE ENDOCHONDRAL BONE IN *SPHENODON*

It has been suggested (Haines, 1938) that the primary function of secondary centres is to allow of the spatial separation of the articular and growth cartilages so that each may be arranged in the form most advantageous in the region concerned. The articular cartilage can then adopt the shape best adapted to the joint structure, and the growth cartilage that best adapted for the formation of the scaffolding of calcified cartilage about which the primary trabeculae of endochondral bone are built. In animals which possess no secondary centres it has been demonstrated, by models for turtles and crocodiles, and in the fossil *Dicynodon* by a polished section, that the main longitudinal trabeculae of the endochondral bone radiate towards the articular cartilage. It remains to show that in *Sphenodon*, the most primitive animal possessing large secondary centres, the trabeculae radiate towards the growth cartilage and not towards the articular cartilage.

In the lower end of the ulna a reconstruction of the bone shows that the trend of the longitudinal trabeculae (*l.t.*) can be recognized distinctly, and that their radiation is towards the growth cartilage. The articular cartilage (see Fig. 2) in this bone has a much sharper curvature than the growth cartilage,

and the trabeculae bear no relation to it. The whole formation would appear to constitute a mechanically efficient structure.

Sphenodon illustrates also a stage in the refinement of the structure of the endochondral bone in the region of its formation. The bays of erosion (*b.e.*) are narrower and more numerous than, for instance, in the turtles, and the primary trabeculae (*p.t.*) correspondingly more numerous and finer in structure. The functional advantage of this refinement of endochondral ossification, which has occurred independently in mammals, birds and reptiles (Lubosch, 1924; Haines, 1938) is unknown, but possibly it is an advantage to have a large number of primary trabeculae available, so that those best placed for the mechanical requirements of the bone may be selected and the remainder destroyed.

The arrangement of the vascular channels which pierce the wall of the shaft has been fully discussed by Heidsieck (1929).

THE MECHANISM OF DEVELOPMENT OF SECONDARY CENTRES

Primary and secondary centres are sharply contrasted in structure. In the primary centres of the long bones the whole width of the central part of the cartilage model becomes hypertrophied, so that the two ends of the cartilage which later form the epiphyses are completely severed from one another by calcified cartilage, and the latter is soon destroyed by erosion from the perichondrium. In the secondary centres, on the other hand, the calcified cartilage occupies only the central parts of the cartilage mass, and is at first, and for a prolonged period of growth, completely cut off from the perichondrium on all sides by a layer of hyaline cartilage (see Fig. 2). This distinction seems to be quite constant, and to apply to all groups of animals having secondary centres, at any rate for the typical centres at the ends of the long bones.

Now even in animals which have no secondary centres the two kinds of calcification are found, the one in the shafts of the long bones, the other in the short bones of the carpus and tarsus. In the turtles for instance, though the primary centres of ossification are of the form familiar in other bony animals, the cartilaginous epiphyses are relatively small and no secondary centres are developed in them. But in the carpal bones of a young animal (Fig. 7) large, well defined secondary centres of calcified cartilage can be found occupying the interior of the cartilage. In the centre figured the structure changes from a large celled region in the centre (*r.l.c.*) through a small celled intermediate region (*r.s.c.*) to a peripheral dàrkly-staining region (*r.d.s.*) where the matrix is more heavily calcified and has a radiate appearance. A thick zone of hyaline cartilage completely surrounds the centre, and cuts it off from the articular fibrocartilage (*a.f.c.*), and from the dense fibro-vesicular tissue (*f.v.t.*) which occupies the region between adjacent skeletal parts where there is no articular cavity, and from the perichondrium on the dorsal and ventral surfaces of the cartilage. The other short bones show a similar structure, and it is only when the

calcification spreads so as to reach the perichondrium that ossification of the centre sets in. Thus from the structural point of view the short and long bones are distinct, and the centres of the short bones resemble the epiphysial centres.

It seems probable, therefore, that in the line leading to *Sphenodon* the cartilages of the ends of the long bones enlarged for the mechanical reasons already

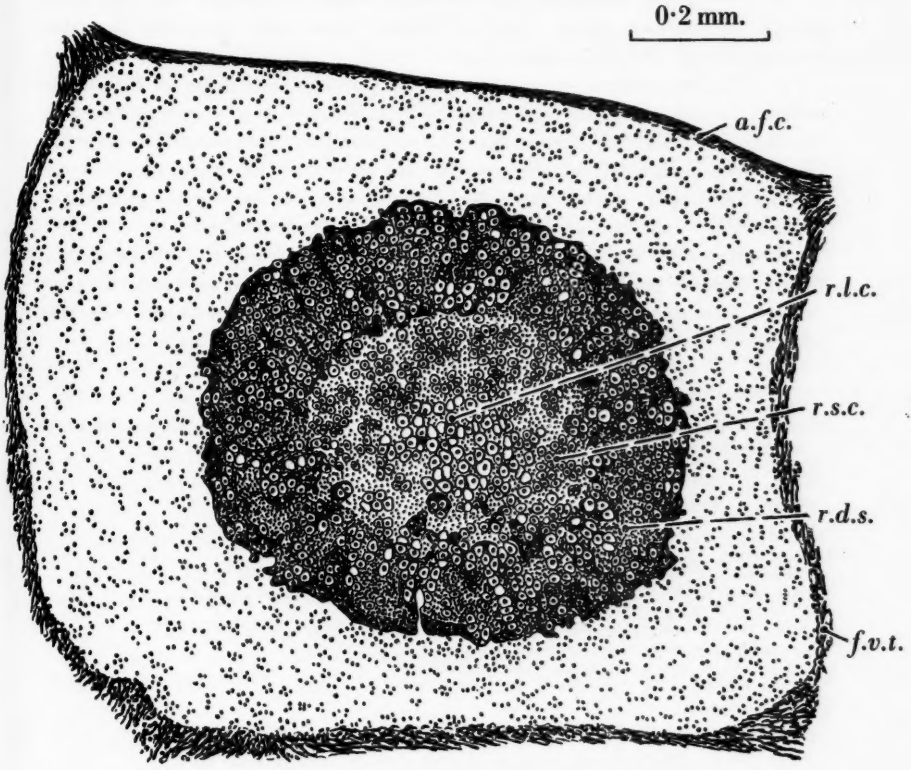

Fig. 7. Section through the second carpal of a young turtle, *Emys orbicularis*. Carapace length 99 mm.

discussed, and that the enlarged cartilages then became influenced by the processes which had already been at work in the short bones for a prolonged geological period. Further, so far as is known, the short bones and the secondary centres have remained similar to each other in each species throughout its evolution. Specializations such as the various modes of ossification and the development of cartilage canals when found in the one are also found in the other, and the time relations of the various stages in development are again similar.

SUMMARY

1. The secondary centres in *Sphenodon* are composed of large masses of calcified cartilage which persist for a prolonged period during growth.

2. These centres are later replaced by marrow and bone, probably derived from the tissues of the shaft which pierce the growth cartilage.

3. The earliest secondary centres were formed of calcified cartilage in all groups of animals which have developed them, and probably persisted throughout the life of the individual.

4. The longitudinal trabeculae of the endochondral bone in *Sphenodon* radiate towards the growth cartilage, and their disposition is compatible with the suggestion that the primary function of secondary centres is associated with the spatial separation of the growth and articular cartilages, so that the arrangement of the trabeculae need not necessarily, as in animals which have no secondary centres, radiate towards the articular cartilage.

5. Secondary centres are formed in the central parts of the cartilages in a manner similar to the centres for the short bones, and it is probable that the earliest secondary centres were formed by mechanisms developed earlier in phylogeny for the ossification of the short bones.

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KEY TO LETTERING

Hyaline cartilage plain, calcified cartilage stippled, bone black

a.c.	articular cartilage	*g.p.b.*	gap in periosteal bone
a.f.c.	articular fibro-cartilage	*h.c.s.c.*	hypertrophied cells of secondary centre
b.e.	bay of erosion	*i.f.c.m.*	isolated fragment of calcified matrix
c.b.m.	cellular bone marrow	*l.t.*	longitudinal trabecula
c.cl.	cartilage column	*m.p.s.*	margin of periosteal shaft
c.c.m.	calcified cartilage matrix	*p.b.s.*	periosteal shaft of bone
c.c.p.b.	calcification near contact of periosteal bone	*p.t.*	primary trabecula
		r.d.s.	darkly-staining region
c.c.s.c.	calcified cartilage of secondary centre	*r.l.c.*	region of large cells
cem.	cement line	*r.s.c.*	region of small cells
c.lg.	cruciate ligament	*s.s.c.*	spaces in secondary centre
con.	contact of periosteal bone with epiphysial cartilage	*v.c.*	vascular channels
		z.f.c.	zone of flattened cells
e.b.	endochondral bone	*z.g.*	zone of growth
e.e.b.	endosteal part of endochondral bone	*z.h.c.*	zone of hypertrophied cells
f.b.m.	fatty bone marrow	*z.u.c.*	zone of undifferentiated cells
f.v.t.	fibro-vesicular tissue		

THE CELL STRUCTURE OF THE MAMILLARY BODY IN THE MAMMALS AND IN MAN

By JERZY ROSE
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INTRODUCTION

It may be reasonably assumed that the mamillary body represents an important structure within the hypothalamus, since a number of large nerve tracts originate or terminate in it. Although there is no lack of descriptions of the architecture of the mamillary body in the principal mammals, most of the authors who have dealt with this subject tend to disregard the comparative anatomical point of view, so that the phylogenesis of this structure and the significance of the individual nuclei remain obscure.

Former degeneration experiments, as made by Gudden (1889), Edinger & Wallenberg (1902), and other authors, have already pointed to the fact that degeneration of the fibre tracts of the mamillary body often produces only a partial degeneration of the nuclei. This leads to the assumption that the nuclei are not single functional units. In this paper the phylogenetic development of the cell structure of the mamillary body has been studied.

Gudden, who made an intensive study of the mamillary body in rodents (mouse), carnivores (cat and dog), monkey and man, distinguished a medial and a lateral nucleus in this structure, the lateral nucleus showing the larger nerve cells. Later he separated off a dorso-anterior and a ventro-posterior section in the medial nucleus of mammals, and stated that in the former the nerve cells are larger and less closely packed than in the latter. In man, Kölliker (1896) described a medial and a lateral nucleus, but in his opinion the medial, unlike that in lower mammals, is not subdivided. In addition, he mentions a nucleus accessorius whose nerve cells are smaller than those in the other two nuclei. In rodents, Cajal (1904) speaks of a medial and a lateral nucleus. The area antero-dorsal to the medial nucleus he terms "foco limitante". Malone (1910) describes in man a medial nucleus with medium-sized nerve cells, which is not subdivided, and a lateral nucleus with large nerve cells which he considers to extend deeply into the region of the infundibulum. The lateral nucleus with its continuation into the infundibulum, Malone calls the n. mamillo-infundibularis. Besides these two nuclei he distinguishes also a small large-celled nucleus situated between the medial and lateral nuclei, which he calls the n. intercalatus.

The findings of Friedemann (1912) in *Cercopithecus* are essentially the same as those of Malone in man. The description of the mamillary body in man as given by Malone has been modified by Foix & Nicolesco (1925), Gagel (1928),

and Greving (1925). Within the medial nucleus Foix & Nicolesco describe two parts, a small-celled inner and a small-celled outer part. By them, Malone's n. intercalatus is termed n. magnocellularis. Greving distinguishes between the ventro-medially situated n. magnocellularis and the dorso-lateral n. parvocellularis. Lateral to these two nuclei stretches the small-celled n. cinereus. The n. intercalatus, identical with that of Malone, is found lateral to this group of nuclei. In man and the dog, Roussy & Mosinger (1935) distinguish a lateral nucleus and two segments within the medial nucleus of other authors.

In the rabbit, whose mamillary body has been subjected to the most detailed investigation, Winkler & Potter (1911) described two nuclei. Nissl divided the ventral posterior portion of the medial nucleus, as described by Gudden, into two parts, a medial with large cells and a lateral with small cells. M. Rose (1935), whose nomenclature has been adopted in this paper, distinguishes in the mamillary body of the rabbit the n. mamillaris medialis seu dispar, n. mamillaris basalis, n. mamillaris intermedius seu papilioformis, n. mamillaris lateralis and n. supramamillaris.

METHODS

The investigation was carried out on paraffin-embedded material and entailed the examination of complete series of frontal sections. The sections, 20 μ in thickness, were stained with cresyl violet.

The volume of the different nuclei was determined as follows: By means of a microprojector the outlines of the nuclei were drawn under a known magnification on cardboard, the thickness of which corresponded to the degree of magnification used. The cardboard was then cut to the shape of the outlined nuclei and the individual pieces pasted together. Each cardboard reconstruction was covered with hominite to prevent swelling in water. The volume of the cardboard reconstruction was then determined by displacement of water from an appropriate container, the displaced water being weighed. The volume of the reconstruction divided by the magnification 3 gives the actual volume of the object. When, for instance, a reconstruction using a magnification of 40 times was made, every second section was reconstructed on cardboard 2 mm. in thickness. Such a cardboard figure thus represented the section magnified 40 times in every direction. Each section was calculated as 25 μ, as it is known from M. Rose's (1928) investigations that the number of sections obtained will be about 20 % less than might be expected from the length of the object.

1. CHIROPTERA

Bat (Synotus barbastellus)

Fig. 1 shows the mamillary body near its rostral extremity. Around the ventricle is a dense collection of nerve cells which, analogous to the nucleus described by M. Rose in the rabbit is called the n. medialis postremus hyp. (*psi*). Lateral to this cell group is a well-defined nucleus with rather loosely packed medium-sized nerve cells, dorso-lateral to which extends an ill-defined region of scattered cells which is adjacent to the pes pedunculi (*pp*) and c. subthalamicum (*cs*). Again, as with similar nuclei in the rabbit, these two nuclei can be called the n. hyp. posterior lateralis (*pl*) and n. hyp.

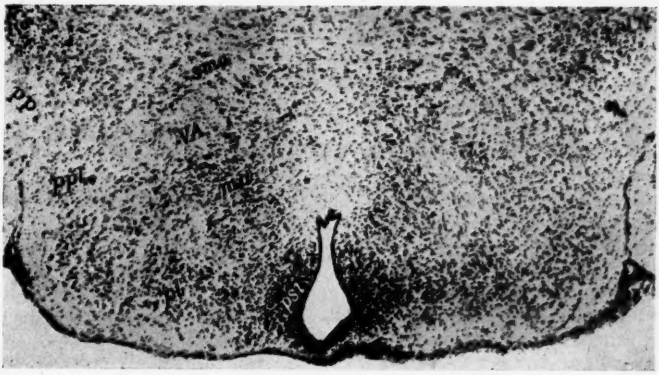

Fig. 1. Bat (*Synotus barbastellus*). N. 4. 207. ×50.

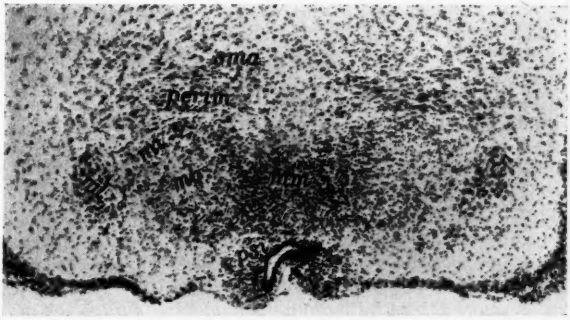

Fig. 2. Bat (*Synotus barbastellus*). N. 4. 215. ×50.

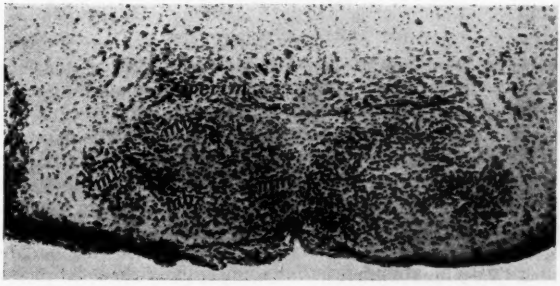

Fig. 3. Bat (*Synotus barbastellus*). N. 4. 219. ×50.

posterior postlateralis (*ppl*). Dorso-lateral to *psi* is a paired nucleus consisting of small, round and medium-sized oval nerve cells, which may be called the anterior mamillary nucleus (*ma*). More caudally the anterior mamillary nucleus passes on directly into the medial mamillary nucleus.

The splitting up of the bundle of Vicq d'Azyr within the anterior mamillary nucleus can be followed in succeeding sections.[1] In Nissl preparations of the bat, however, it is rather difficult to trace this bundle and the fornix.

Above the anterior mamillary nucleus, covering it like a roof, extends the supramamillary nucleus (*sma*) consisting of loosely packed nerve cells which are larger and more deeply staining than those of the anterior mamillary nucleus. There is a partial fusion of the supramamillary nucleus from each side in the mid-line. The supramamillary nucleus does not stand out sharply from the other nuclei.

Fig. 2 shows the mamillary body fully developed. Parts only of the n. medialis postremus hyp. (*psi*) are left, the n. hyp. posterior lateralis (*pl*) and n. hyp. posterior postlateralis (*ppl*) having disappeared. The mamillary body has moved ventrally to the base. In place of the anterior mamillary nucleus (*ma*) an unpaired nucleus has arisen which is the medial mamillary nucleus (*mm*). The nerve cells of the latter nucleus (*mm*) are seen as round, closely packed, granular cells. Ventro-lateral to the medial mamillary nucleus lies the basal mamillary nucleus (*mb*), whose nerve cells, whilst being more loosely arranged, resemble those of the medial mamillary nucleus. On the lateral margin of the basal mamillary nucleus (*mb*) the nerve cells condense into a band which stretches from its base to the lateral aspect of the medial mamillary nucleus, forming a semicircle around the basal mamillary nucleus. In rodents, as will be shown, it is very well developed. It may be termed the intermediate mamillary nucleus (*mi*). Lateral to the medial group of nuclei a new nucleus has appeared, the lateral mamillary nucleus (*ml*). It is an oval, sharply circumscribed nucleus containing relatively large and deeply staining nerve cells. The supramamillary nucleus (*sma*) extends dorsally, and ventral to it is a sharply defined band composed of deeply staining, elongated nerve cells, which enlarge caudally. This part of the supramamillary nucleus will be called the perimamillary nucleus (*perim*).

Fig. 3. The ventricle has disappeared; the medial mamillary nucleus has diminished, but the basal mamillary nucleus (*mb*) is seen distinctly. The lateral mamillary nucleus (*ml*), still large, is about to disappear, its nerve cells intermingling with those of the intermediate mamillary nucleus (*mi*). The perimamillary nucleus is prominently developed, and its large deeply staining nerve cells are arranged in rows, a characteristic of this nucleus. Compared with the supramamillary nucleus the perimamillary nucleus is also far better defined. In the following five sections the mamillary body disappears altogether.

2. INSECTIVORES

(*a*) *Mole* (Talpa europaea)

Fig. 4 shows the mamillary body fully developed. The n. medialis postremus hyp. (*psi*) is about to disappear. Lateral to *psi* are the remains of a nucleus which is termed the premamillary nucleus (*pre*), and which, as a rule, is found in most mammals immediately in front of the rostral extremity of the mamillary body. It is composed of numerous small nerve cells together with a few large deeply staining cells. As will be shown later, this nucleus is not homologous with the premamillary nucleus described by M. Rose in the rabbit.

Dorso-lateral to the premamillary nucleus (*pre*) is the anterior mamillary nucleus (*ma*). In the mole, it forms a very large round dense nucleus. The nerve cells are of medium size with an abundance of cytoplasm, and the cell processes are quite

[1] In all the figures the thalamo-mamillary tract and the fibre tracts joining it are given the common name of the bundle of Vicq d'Azyr.

distinct. In Fig. 4 both anterior mamillary nuclei are already connected to the medial mamillary nucleus (*mm*). In the rostral part of the mamillary body, however, only the two anterior mamillary nuclei (*ma*) are present, being sharply distinguished medially from the premamillary nucleus which has taken the place of the medial mamillary nucleus (*mm*). The medial mamillary nucleus is less dense than the anterior (*ma*), its nerve cells being somewhat smaller, rounded and staining less intensely. Dorsally the nerve cells condense, clearly giving rise to a band of more deeply staining large cells. Above the medial mamillary nucleus the supramamillary nucleus is distinctly visible. It forms a large, loose, well-defined nucleus. Its

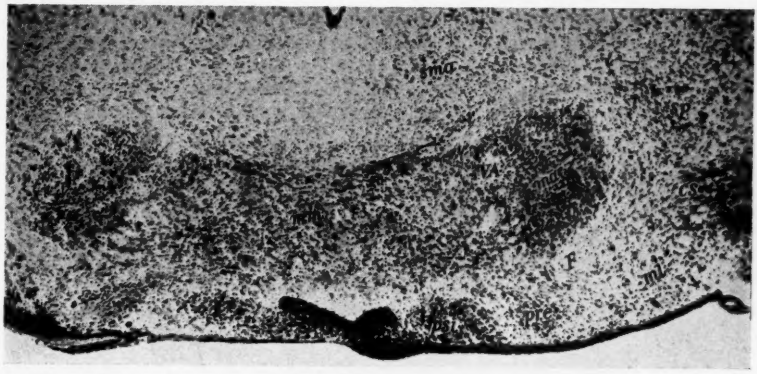

Fig. 4. Mole (*Talpa europaea*). Kt. 384. ×50.

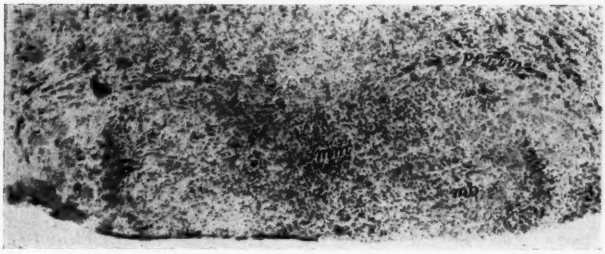

Fig. 5. Mole (*Talpa europaea*). Kt. 394. ×50.

nerve cells are of small or medium size, and the cell processes are abundant. Ventrolateral to the fornix an accumulation of large deeply staining nerve cells is seen, but is not sharply defined. This corresponds most probably to the lateral mamillary nucleus (*ml*), as may be inferred from its typical position as well as from the form and staining of the nerve cells.

Fig. 5. The mamillary body here forms the base of the hypothalamus. The anterior mamillary nucleus on each side has already disappeared. The medial mamillary nucleus shows a distinct condensation of nerve cells in its centre. Ventrolateral to the medial mamillary nucleus is a loosely packed nucleus with smaller nerve cells which corresponds to the basal nucleus (*mb*). The intermediate mamillary nucleus (*mi*) arises lateral to the basal nucleus (*mb*), as a narrow band which is

denser than the basal nucleus (*mb*), though its nerve cells are similar to those of that basal nucleus. Dorsally, on both sides, the intermediate mamillary nucleus (*mi*) fuses with the medial mamillary nucleus (*mm*).

Generally, the differences between the medial mamillary nucleus (*mm*), the basal nucleus (*mb*) and the intermediate mamillary nucleus (*mi*) are not very distinct, particularly caudally, where the nerve cells become scattered so that it becomes difficult to distinguish between the medial mamillary nucleus and the basal mamillary nucleus.

Dorsal to the medial nuclear group, the perimamillary nucleus (*perim*) has taken the place of the supramamillary nucleus (*sma*). In the mole it is enormously developed. The perimamillary nucleus is composed of several rows of large, elongated, oval or polymorphous deeply staining nerve cells with numerous cell processes. It is more sharply demarcated from the other nuclei than the supramamillary nucleus. Within a short distance the perimamillary nuclei fuse.

Within the next twenty sections the mamillary body disappears completely.

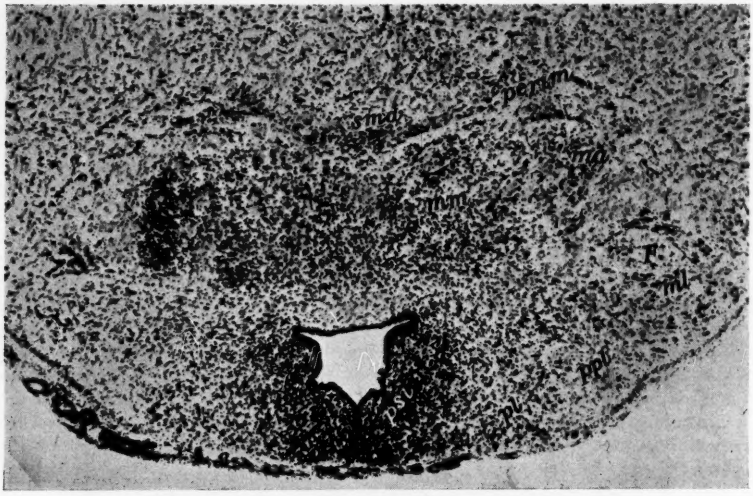

Fig. 6. Hedgehog (*Erinaceus europaeus*). J. 625. ×40.

(*b*) *Hedgehog* (Erinaceus europaeus)

Fig. 6 represents a section from the centre of the mamillary body. The n. medialis postremus hyp. (*psi*), still large, is situated around the ventricle, whilst lateral to it are remains of the nuclei *pl* and *ppl*. The unpaired medial nucleus, which seems to originate from the fusion of the nuclei of both sides, consists of medium-sized nerve cells, round or oval in shape, with a narrow border of cytoplasm and poorly visible cell processes. Lateral to the medial mamillary nucleus (*mm*) the anterior mamillary nucleus (*ma*) is attached to it, being distinguished by smaller and more closely packed nerve cells. The anterior mamillary nucleus is already about to disappear on the right side.

In the hedgehog, although it can readily be seen, the anterior mamillary nucleus (*ma*) is much less developed than in the mole. Its medium-sized, polygonal, closely packed nerve cells show abundant but poorly staining cytoplasm. Above the anterior

mamillary nucleus (*ma*), the supramamillary nucleus develops to the same large size as in the mole. The supramamillary nucleus (*sma*) consists of closely packed nerve cells, both of medium and large size, which are predominantly elongated and oval in shape, with an abundance of clearly visible cytoplasm. The cell processes are broad but can only be followed for a short distance. The supramamillary nuclei show a number of very large nerve cells, and fuse in the mid-line. Lateral to the supramamillary nucleus, the perimamillary nucleus (*perim*) has already appeared, and lateral to the fornix the lateral mamillary nucleus (*ml*) has developed. The latter is much more distinct than in the mole; that it is homologous with the lateral mamillary nucleus of rodents seems beyond doubt. The nerve cells of the lateral mamillary nucleus are large, mainly polymorphous, the cytoplasm being abundant and the cell processes distinctly visible. Both the cytoplasm and the cell nuclei stain intensely.

Fig. 7 shows the mamillary body farther caudally. The medial mamillary nucleus has disappeared almost completely. Its place has been taken by the loosely packed basal mamillary nucleus (*mb*), whose cells are much smaller and less deeply

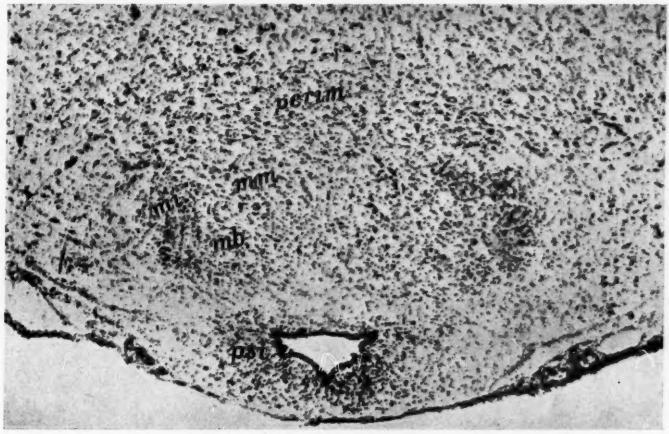

Fig. 7. Hedgehog (*Erinaceus europaeus*). J. 636. ×40.

staining than those of the medial mamillary nucleus, whilst they are rounded and show a narrow border of cytoplasm. Lateral to the basal mamillary nucleus (*mb*) a conspicuous accumulation of nerve cells of the same type is found. This is probably the intermediate mamillary nucleus (*mi*). Dorso-laterally the intermediate mamillary nucleus encircles the basal mamillary nucleus (*mb*) and disappears into the remains of the medial mamillary nucleus. Caudally the intermediate mamillary nucleus (*mi*) becomes still larger. In the rostral parts, however, separation of the intermediate and anterior mamillary nuclei is difficult, as the nerve cells of each show no marked differences.

Dorsally, the perimamillary nucleus has displaced the supramamillary nucleus. It is enormous, its nerve cells being large, elongated and deeply staining with an abundance of cytoplasm. The perimamillary nucleus covers the mamillary body dorsally, and partially on its lateral aspect. The lateral margin of the perimamillary nucleus shows larger cells than the medial. Fusion of both perimamillary nuclei takes place in the mid-line.

3. RODENTIA

(*a*) *Squirrel* (Sciurus vulgaris)

Fig. 8 shows the rostral extremity of the mamillary body. The n. medialis postremus hyp. (*psi*) is visible around the broad ventricle, and to the right a portion of the premamillary nucleus is still present. In the centre of the figure the unpaired anterior mamillary nucleus is seen. Its medium-sized cells are fairly dense. The cytoplasm is readily visible and the cell processes are abundant. The fibres of the bundle of Vicq d'Azyr are distinctly seen penetrating the anterior mamillary nucleus (*ma*). Dorso-lateral to the fornix, the sharply defined oval basal mamillary nucleus can be seen, the cells of which are large, deeply staining, and show numerous cell processes. Above the anterior mamillary nucleus the extremity of the supramamillary nucleus is visible.

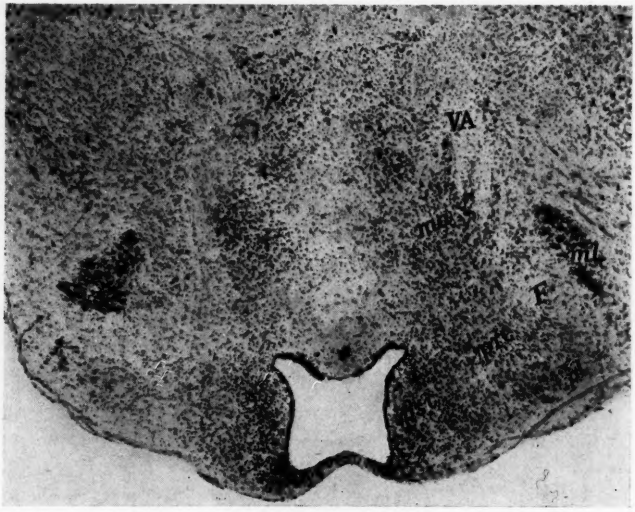

Fig. 8. Squirrel (*Sciurus vulgaris*). W. 1. 513. ×30.

Fig. 9. The mamillary body has moved to the base. In place of the anterior mamillary nucleus, the unpaired large medial nucleus (*mm*) appears. Its cells are closely packed, resembling those of the anterior mamillary nucleus. It is obvious that the medial mamillary nucleus has originated from the fusion of the nuclei of both sides. At the lateral aspect of the medial mamillary nucleus the cells are more scattered. Ventral to the medial nucleus, a collection of considerably smaller nerve cells can be seen forming the beginning of the basal nucleus (*mb*). Dorsally in the figure, the well-developed, large, supramamillary nucleus is seen, forming a roof over the mamillary body. Its cells stain well, and consist of small and also rather large types. The small cells are rounded, whilst the large are angular or polymorphous with an abundance of cytoplasm. Both supramamillary nuclei fuse in the mid-line. This fused portion consists almost exclusively of small nerve cells.

Fig. 10. The medial mamillary nucleus shows a considerable decrease in size, while the basal nucleus (*mb*) has increased. The latter consists of loosely packed, small, feebly staining cells. There is a partial fusion of both basal nuclei (*mb*). From the base of the basal nucleus, the intermediate mamillary nucleus (*mi*) develops,

being composed of cells similar to those of the basal nucleus though more closely packed. The intermediate mamillary nucleus (*mi*) encloses the medial mamillary nucleus (*mm*). Fusion of both intermediate nuclei takes place in the mid-line dorsally. This fused portion, which consists of larger and somewhat more deeply staining nerve cells than those of the individual nuclei, is called the central mamillary nucleus (*mc*), in the rabbit by M. Rose.

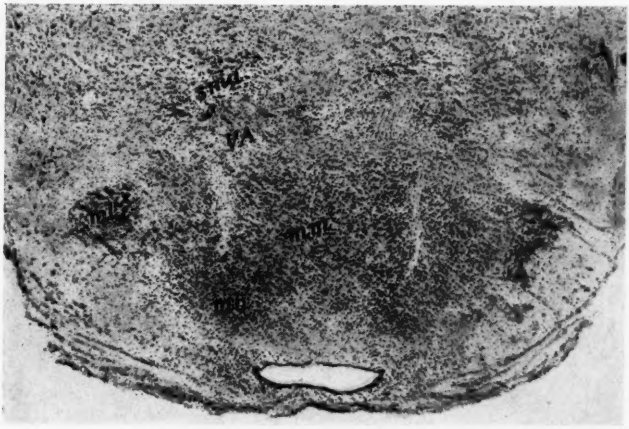

Fig. 9. Squirrel (*Sciurus vulgaris*). W. 1. 527. ×30.

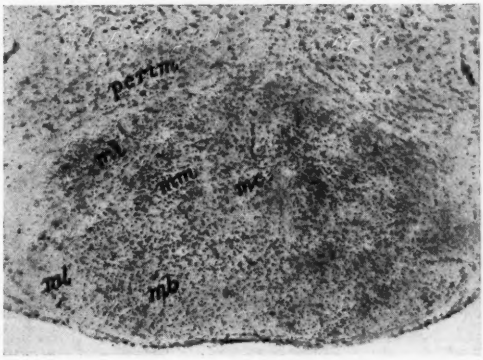

Fig. 10. Squirrel (*Sciurus vulgaris*). W. 1. 547. ×30.

Of the lateral nuclei, only isolated nerve cells are visible. Dorsal to the medial group of nuclei the perimamillary nucleus has arisen in place of the supramamillary nucleus. In the squirrel it is poorly developed. The characteristically arranged rows of elongated and loosely packed nerve cells, however, can easily be recognized.

Caudally the basal nuclei are the only elements representing the medial nuclear group.

(*b*) *Rabbit* (Lepus cuniculus)

The structure of the mamillary body in the rabbit requires no detailed description. It is essentially identical with that of the squirrel, and detailed descriptions and illustrations of its structure have already been given by M. Rose. It may be noted that the nucleus, which in the rabbit, insectivores and the bat I have called the anterior mamillary nucleus, is not considered by M. Rose to belong to the mamillary body, and in the rabbit he has described it as the premamillary nucleus. This may depend on the fact that in the rabbit the close connexion of the mamillary body with the bundle of Vicq d'Azyr is not so obvious and conspicuous as in the squirrel (see Fig. 8).

Regarding quantitative differences in the development of the different nuclei in the rabbit compared with the squirrel, the following may be mentioned: The perimamillary nucleus is even less developed. Only by comparative anatomical investigation can the perimamillary nucleus in the rabbit be regarded as a distinct entity. The lateral nuclei are relatively larger. The intermediate nuclei are particularly well developed and can easily be distinguished from the other nuclei.

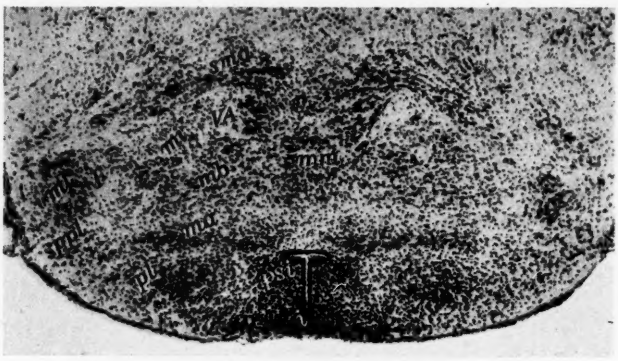

Fig. 11. Mouse (*Mus musculus*). My. 1. 318. ×50.

(*c*) *Mouse* (Mus musculus)

Fig. 11. The n. medialis postremus hyp. (*psi*) is situated round the ventricle, whilst the n. infundibularis (*i*) is ventral to this. Lateral to *psi* extend the well-known nuclei *pl* and *ppl* (nn. hyp. posterior lateralis and postlateralis), dorsal to which is the anterior mamillary nucleus (*ma*). In the mouse the anterior mamillary nucleus is represented by a large oval nucleus which, in rostral sections, runs, as in other rodents, dorso-medially to ventro-laterally. It does not disappear, however, as in other rodents, immediately after the medial mamillary nucleus has arisen, but is displaced downwards. Therefore, after the chief nuclei of the mamillary body have appeared it extends ventral to them as a narrow band, being separated from them by a narrow acellular zone. The anterior mamillary nucleus is fairly dense, its cells being of medium size, oval or angular, and staining well. Both fuse partially in the mid-line.

In the centre of the figure the medial mamillary nucleus (*mm*) can be seen. It is relatively small but dense, and its cells are round or angular and relatively large. Ventro-laterally the basal nucleus (*mb*) is well defined, its nerve cells are more scattered than those of the medial nucleus (*mm*), besides being smaller and mostly round. The

cell processes are scarcely visible. Dorso-lateral to the basal nucleus (*mb*), the intermediate nucleus (*mi*) appears, being well developed in the mouse. Its nerve cells are more closely packed and somewhat smaller than in the basal nucleus (*mb*). The medial margins of the intermediate nuclei, as in other rodents, show larger and more deeply staining cells. In the mouse it is not always easy to distinguish the nerve cells of the medial margin of the intermediate nucleus from those of the supramamillary nucleus. Dorsal to the medial nucleus (*mm*), the supramamillary nucleus is found large and well developed. Its structure shows much resemblance to that of the supramamillary nucleus in the squirrel. The cells are large and deeply staining. In the mid-line the nuclei fuse, the cells in this area being smaller and less deeply staining.

Lateral to the fornix the lateral mamillary nucleus (*ml*) has developed and is almost round. Its cells are round or angular, deeply staining, and show an abundance of cytoplasm.

More caudally than in Fig. 11, both medial and basal nuclei (*mm* and *mb*) become smaller, whereas the intermediate nucleus (*mi*) increases considerably in size and extends as a broad band dorso-medially. In the mouse (as well as in the rabbit and squirrel) the large-celled part of the intermediate nucleus (*mi*) is very well seen. These parts of the intermediate nucleus on each side fuse and, forming a central nucleus, pass on to the medial nucleus.

Dorsally, the perimamillary nucleus (*perim*) has arisen in place of the supramamillary nucleus (*sma*). In the mouse, as in all the investigated rodents, the perimamillary nucleus is poorly developed, but, nevertheless, can be seen clearly. It is narrow, sharply defined and closely attached to the mamillary body, and consists of a few rows of flattened, large, deeply staining nerve cells.

4. CARNIVORA

(*a*) *Polecat* (Mustela putorius)

The mamillary body of the polecat differs considerably from that of the other mammals already described. Its most significant feature is the disappearance of the unpaired nucleus which is present in rodents and insectivores, as well as in the bat.

Fig. 12 shows the medial section of the mamillary body. The ventricle and the nuclei surrounding it have just disappeared. Laterally (to the left) can be seen a narrow band of large deeply staining nerve cells. This nucleus probably corresponds to Grünthal's (1929) nucleus 3 in the dog. The anterior mamillary nucleus (*ma*) is very well seen, and within a short distance a roof-shaped portion (ma_1) can be demarcated. The cells of the anterior nucleus (*ma*) are fairly large, show a dark round or oval nucleus and an abundance of poorly visible cytoplasm, whilst area ma_1 is composed of smaller and more closely packed nerve cells. There is a partial crossing of both anterior nuclei in the mid-line but no distinct fusion. Below, but not sharply defined from the anterior mamillary nucleus (*ma*), is the medial mamillary nucleus (*mm*), into which the bundle of Vicq d'Azyr passes. The medial mamillary nucleus is paired and does not fuse with the medial nucleus of the opposite side, but is always separated from it by a raphe. The cells of the medial nucleus (*mm*) are somewhat smaller than those of the anterior, and the arrangement of the cells is looser. At the dorso-lateral margin the cells are generally more closely packed and slightly larger than in the centre.

With cresyl violet the background stains a deep reddish purple, darker than in the anterior nucleus, so that in the preparation it is considerably easier to distinguish these nuclei than it is in the figure. Ventral to the medial nucleus is the basal nucleus (*mb*), its cells being much smaller and less deeply staining than those of the medial nucleus. The basal nucleus contains many glia-like cells. Lateral to the medial nuclear group the lateral nucleus (*ml*) is seen. It is oval in shape and enormously

developed, and its cells, as in the other animals, are large, deeply staining and provided with numerous cell processes.

Above the anterior nucleus (*ma*) the very well-developed supramamillary nucleus (*sma*) is visible. It covers the mamillary body as a broad band in its typical roof-like manner. Its nerve cells are rather large, deeply staining and loosely arranged.

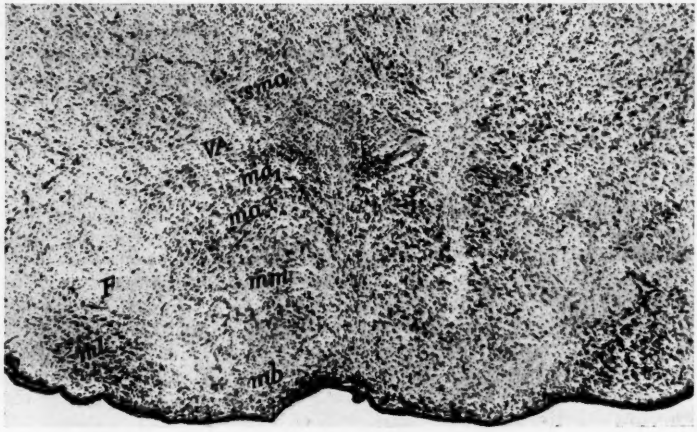

Fig. 12. Polecat (*Mustela putorius*). Tch. 3. 764. ×35.

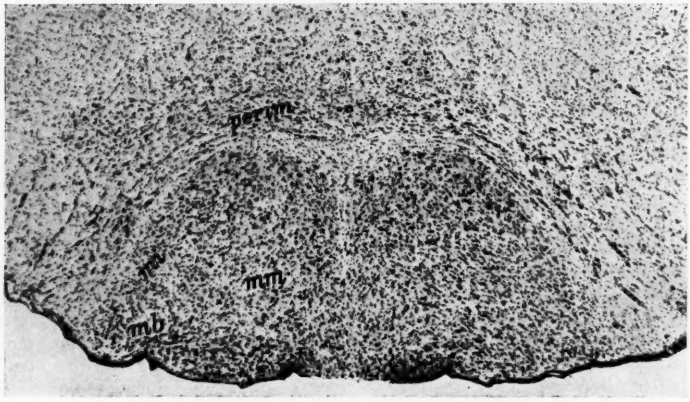

Fig. 13. Polecat (*Mustela putorius*). Tch. 3. 795. ×35.

The thalamo-mamillary tract often divides the supramamillary nucleus into two parts. There is a mingling of the cells of both supramamillary nuclei in the mid-line, whilst they are not sharply demarcated from the other nuclei.

Fig. 13 shows the mamillary body more caudally. The anterior and lateral nuclei have completely disappeared. The medial nuclear group is enormously developed and is surrounded on all sides by a medullary capsule. The deep staining of

the background of the medial nucleus is well seen. Ventro-latero-basal to the medial nucleus (*mm*), the basal nucleus (*mb*) can be recognized by its small, round, feebly staining cells. From the basal nucleus a narrow band of cells, resembling those of this nucleus, becomes separated. It is evidently homologous with the intermediate nucleus (*mi*) of lower animals, but a distinction from the basal nuclei can be made with ease only in some places.

Dorsally, the perimamillary nucleus (*perim*) has appeared in place of the supramamillary nucleus. The perimamillary nucleus surrounds the mamillary body above and on the sides, like a band. It is composed of several rows of elongated, large, deeply staining cells. It shows a distinctly greater development than in rodents.

In the further sections of the medial nuclear group, apart from the perimamillary nucleus, only the medial nucleus is left. Farther caudally, however, the cells of the medial nucleus are more loosely packed. Within about 40 sections caudal to Fig. 13, the mamillary body disappears completely.

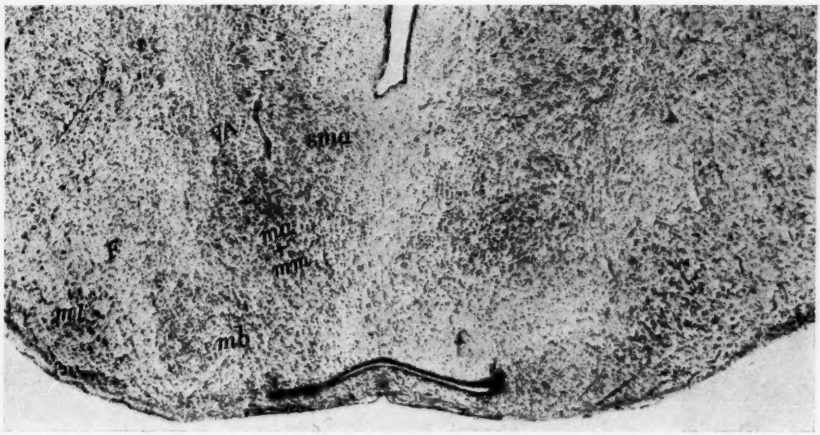

Fig. 14. Dog (*Canis familiaris*). P. 4. 185. ×25.

(*b*) *Dog* (Canis familiaris)

Fig. 14 shows the rostral end of the mamillary body. Ventro-laterally in the figure, Grünthal's nucleus 3 can be seen. Above the ventricle, on both sides, the anterior nucleus (*ma*) is encountered, whilst to the left and ventral to *ma* the basal nucleus (*mb*) is seen and will be described later.

The anterior nucleus is paired and composed of large, deeply staining cells mostly oval in shape. The cytoplasm is abundant and stains well, whilst the cell processes can be traced for long distances. Generally, the cells of the anterior nucleus are somewhat larger and more closely packed than those of the medial nucleus (*mm*) which appears farther caudally. The differences in the dog, however, being slight, the anterior and the medial mamillary nuclei are given the same name.

The well-developed lateral nucleus (*ml*) on each side is situated between the ventricle and nucleus 3, ventro-lateral to the fornix. The cells of the lateral nucleus are large with abundant deeply staining cytoplasm. This nucleus, however, is somewhat less well defined than in the polecat.

Above the medial group of nuclei the supramamillary nucleus has just appeared and will be described in the next figure.

Fig. 15. The lateral nucleus (*ml*) has already gone completely. The anterior and medial nuclei have enlarged considerably, and they are paired. A triangular-shaped collection of small elongated cells unites both the anterior nuclei dorsally. Ventrolateral to the anterior and medial nuclei the basal nucleus (*mb*) becomes obvious. Its cells are more scattered than those of *ma* and *mm*, and are small and less deeply staining. The border of cytoplasm too, is much narrower, whilst the large nucleus of the cells is remarkably light and has an inflated appearance. The cell processes are thin and on the whole readily visible. Lateral to the basal nucleus (*mb*) the intermediate nucleus (*mi*) appears but is poorly developed.

Above the medial nuclear group the supramamillary nucleus is well developed. It covers the mamillary body in its typical roof-like manner. Its elongated oval cells are large and loosely packed, with abundant deeply staining cytoplasm and light oval nuclei. The cell processes are thick and very distinct. The supramamillary nucleus is interrupted by the fibre bundles coursing into the mamillary body.

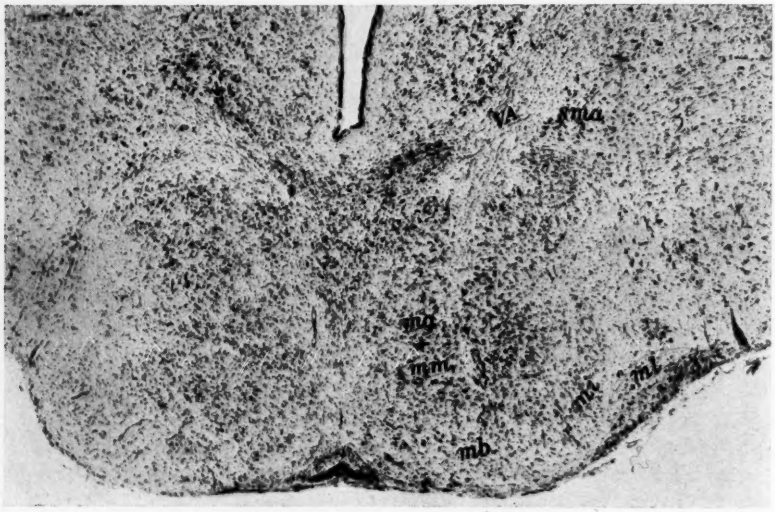

Fig. 15. Dog (*Canis familiaris*). P. 4. 208. ×25.

More caudally the combined anterior and medial nucleus becomes more loosely packed so that it is less sharply defined from the basal nucleus (*mb*). Still farther caudally, the whole of the medial group of nuclei becomes more and more deficient in cells. Caudally, too, the supramamillary nucleus is displaced by the perimamillary nucleus. In the dog the perimamillary nucleus is only incompletely developed. The arrangement of the nerve cells in rows, together with a looser type of formation than in the supramamillary nucleus, however, can be recognized, and the cells are seen to be somewhat elongated, medium-sized and deeply staining.

5. PRIMATES

(*a*) *Rhesus monkey* (Macaca mulatta)

Fig. 16 represents the rostral extremity of the mamillary body. The medial mamillary nucleus (*mm*) occupies the greater part of the figure. In the monkey, the anterior mamillary nucleus (*ma*) cannot be distinguished from the medial mamil-

lary nucleus, and at no point does fusion of the latter nucleus take place with its fellow of the opposite side. Its cells are of medium size and not sharply defined. The background has a dark reddish tinge. The cytoplasm of the cells is abundant but poorly visible, whilst the round or oval nucleus is large. Ventro-laterally, the basal nucleus (*mb*) is seen. Here the ground substance is considerably lighter, the cells are round and small (much smaller than in the medial nucleus), are loosely packed, and show a narrow rim of cytoplasm. There is also an abundance of glia cells. On the lateral margin of the basal nucleus (*mb*) the small nerve cells condense into a narrow band so that they may be spoken of as an intermediate mamillary nucleus (*mi*), but as in the dog it is only feebly developed.

Lateral to the medial nuclear group the lateral nucleus (*ml*) is seen. It is almost round, is sharply demarcated, and its position and composition are typical. The cells are large with an abundance of cytoplasm and deeply staining cell processes.

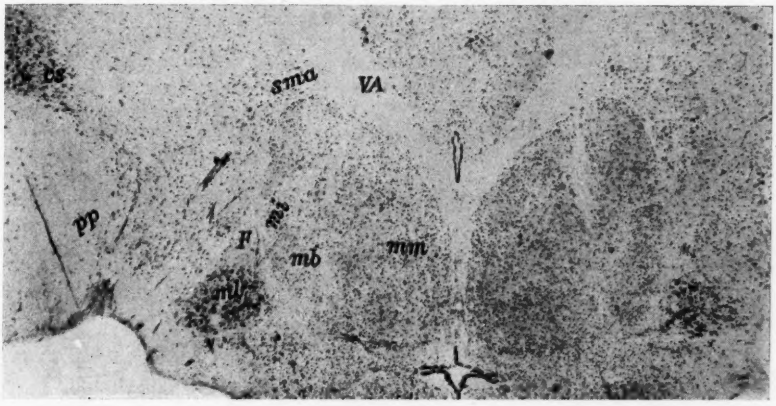

Fig. 16. Macaque monkey (*Macaca mulatta*). M. 2. ×20.

Above the medial nucleus (*mm*) the supramamillary nucleus is seen to begin, and farther caudally becomes fully developed. The supramamillary nucleus covers the mamillary body in its usual roof-like manner and is composed of medium-sized, loosely packed, deeply staining nerve cells. Caudally, a rather large perimamillary nucleus is seen, closely attached to the mamillary body. It stretches as a band, with its maximum width laterally, to surround the mamillary body almost as far as the ventral surface. Its cells, elongated and well-staining, are about the same size as those in the supramamillary nucleus.

Caudally, the medial nuclear group, too, becomes altered because on the medial margin and the upper half of the lateral margin of the nucleus (*mm*) a very narrow zone is seen consisting of more closely packed cells resembling those of the medial nucleus. It is missing only at the junction of the medial and basal nuclei (*mm* and *mb*). Still farther caudally the intermediate and basal nuclei (*mi* and *mb*) disappear completely, and of the medial group of nuclei only the medial nucleus is left.

About 35 sections caudally to Fig. 16 the mamillary body disappears completely.

(*b*) *Man*

Owing to the size of the microphotograph the mamillary body is discussed in relation to a series of sections prepared from the brain of a 3-months-old child. These did not differ from a similar series in the adult.

The structure of the mamillary body in man is very similar to that of the rhesus monkey. There is no fusion of the medial nuclei. The nuclear group on each side forms the well-known eminence which can be seen macroscopically.

Fig. 17 shows the rostral extremity of the mamillary body, and at this level both the medial and basal mamillary nuclei are encountered. The medial nucleus (*mm*) consists of medium-sized, well-staining, round or polygonal cells with abundant cytoplasm, whilst the ground substance shows a reddish tinge. Ventro-laterally, the basal nucleus (*mb*) is readily seen. As in the monkey, it consists of cells which are

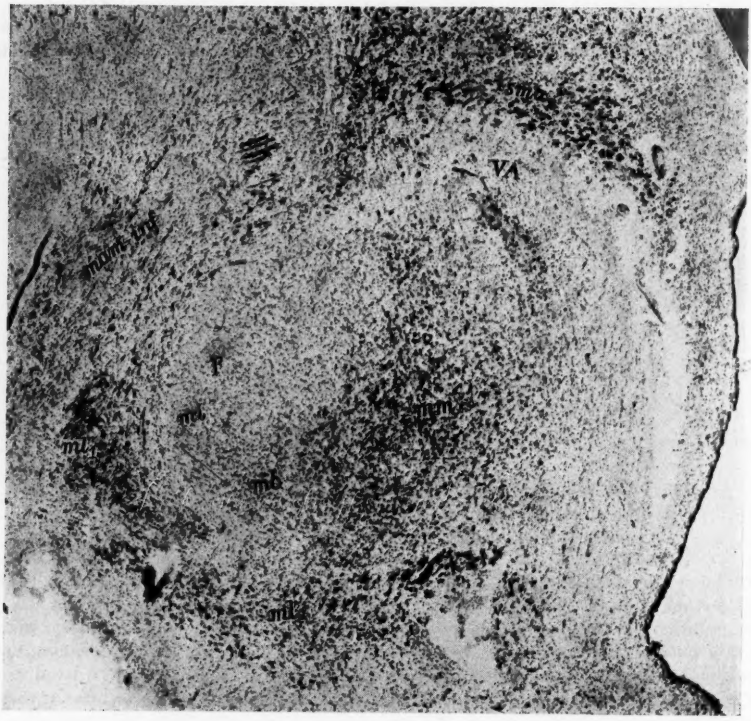

Fig. 17. Man. H. 45. 659. ×30.

much smaller, rounder and more scattered than those of the medial nucleus (*mm*), whilst glia-like cells are also present.

Lateral to the basal nucleus (*mb*), a narrow band of nerve cells appears which may be considered homologous with the intermediate nucleus (*mi*) of lower mammals.

Ventral and lateral to the medial nuclear group, the lateral nucleus (*ml*) is distinctly visible, as in most mammals, although it is divided into two parts. The position and structure of the lateral nucleus is absolutely typical. Its cells are large, polymorphous and deeply staining.

Above the bundle of Vicq d'Azyr the supramamillary nucleus, clearly developed, extends in its typically roof-like manner. It consists of large, deeply staining, polygonal nerve cells.

Farther caudally the medial nucleus attains its full development. Only traces of the lateral nucleus (*ml*) are left, whilst both the basal and intermediate nuclei (*mb* and *mi*) are much reduced.

The supramamillary nucleus quickly decreases in size and is interrupted by fibre tracts. Thus the medial nucleus (*mm*) forms the chief part of the mamillary body. Proceeding farther caudally the mamillary body is represented by the medial nucleus only, and is enclosed on all sides by a medullary capsule. Some rows of loosely packed, elongated nerve cells are closely attached to the capsule and obviously represent the perimamillary nucleus in man.

It may be noted that the lateral mamillary nucleus corresponds not only to Malone's n. intercalatus, but also forms part of his n. infundibulo-mamillaris. Probably Malone's n. intercalatus corresponds only to the nucleus which in Fig. 17 is indicated as ml_1.

According to Malone's description, ml_2 would correspond only to an expansion of the n. infundibulo-mamillaris.[1] From the comparative anatomical aspect, however, one is entitled to regard these parts as homologous with the lateral mamillary nucleus of lower mammals. Malone's nucleus infundibulo-mamillaris is composed of several nuclei which, from a comparative anatomical point of view, are different. The following nuclei, at least, enter into its formation: the n.n. perifornicatus, supra-fornicatus and premamillaris, rostral to the mamillary body; and the supramamillary nucleus, part of the lateral mamillary nucleus and the nucleus, which in Fig. 17 is indicated as the infundibulo-mamillary nucleus (n. mamillo-infundibularis), in the mamillary body itself.

Greving's n. magnocellularis corresponds to the medial mamillary nucleus as described in man and mammals, his n. parvocellularis to the basal mamillary nucleus (*mb*), and the nucleus intercalatus to the lateral mamillary nucleus. Possibly Greving's n. cinereus corresponds to the intermediate mamillary nucleus.

DISCUSSION ON THE STRUCTURE OF THE MAMILLARY BODY FROM THE COMPARATIVE ASPECT

In the mammals which have been investigated for the purpose of this paper, the nuclei may be divided into three groups which are common to all: medial, lateral and dorsal.

(*a*) *The medial group of nuclei*

The main part of the mamillary body is nearly always formed by this group, and, in regard to the structure of its individual nuclei in the different orders, it shows the greatest variations. The thalamo-mamillary tract terminates in it.

In the bat and in rodents this nuclear group shows a similar structure, consisting of an anterior paired nucleus (*ma*), a medial unpaired nucleus (*mm*), and very clearly developed basal and intermediate nuclei (*mb* and *mi*). In all mammals the basal nucleus lies ventral or ventro-lateral to the medial nucleus, and is composed of smaller and less closely packed cells than the medial. The intermediate nucleus in all mammals shows the same cellular type as the basal nucleus, though its cells are more closely packed. It always arises as a narrow

[1] There is good reason for most authors (Greving, Grünthal and others) identifying Malone's n. intercalatus with the lateral mamillary nucleus as described here. This supposition, however, does not accord with Malone's description and drawings.

band, and, when it is well developed, surrounds the medial nucleus on its lateral side. In the insectivores the anterior nucleus has reached a high development. In the mole it is especially well marked, but not to the degree seen in the hedgehog. It may be inferred, though probably it is not absolutely certain, that the anterior mamillary nucleus in insectivores is homologous with the element of the same name in the bat and rodents.

In the hedgehog and mole the paired medial nucleus is very well developed. Though the basal and intermediate nuclei are undoubtedly present, they are very much less differentiated than in rodents and bats.

In carnivores a modification in the structure of the medial nuclear group can be observed. In the polecat the anterior mamillary nucleus may still be defined quite easily, but in the dog it cannot be clearly separated from the medial nucleus.

In addition, the unpaired nucleus disappears in carnivores as well as in the higher mammals. In its place a large paired nucleus develops, which in the polecat is most probably homologous with the unpaired nucleus of rodents, insectivores and bats. In the polecat the nucleus is large, whilst in the dog, where as already stated it cannot be separated with certainty from the anterior nucleus, it already forms the chief part of the mamillary body.

In carnivores the basal and intermediate nuclei (especially the latter) show a regression in development, so that the characteristic appearance of the mamillary body, in rodents, bats and (partly) in insectivores, is altered appreciably.

In the dog, appearances resembling those seen in the monkey and man are found. The cell structure of the mamillary body in the polecat forms a link between the lower and higher mammals.

In the monkey, an anterior mamillary nucleus cannot be defined. Perhaps the condensation of nerve cells on the dorso-medial and partially on the lateral margins of the medial mamillary nucleus may be taken as representing remnants of the independent anterior mamillary nucleus. The similar position of the anterior mamillary nucleus to the medial nuclei of the polecat would favour this assumption.

A further regression has occurred in the basal and intermediate nuclei, the largest nucleus in the monkey being represented by the medial mamillary nucleus.

In man, conditions similar to those in the monkey are found. The medial nucleus is large, the basal nucleus is much reduced, and only traces of the intermediate can be seen, whilst nothing can be found to indicate the anterior nucleus. Thus, from what has been stated, it is probable that in man and monkey the medial nucleus corresponds to the combined anterior and medial nuclei of rodents, insectivores and bats. In the dog this fusion can be seen taking place, but in the polecat they are still readily distinguishable from one another.

In all mammals the basal and intermediate nuclei can be distinguished

throughout, but they show a definite regression from the carnivores upwards.

Attention may be drawn to the staining reaction of the medial nuclear group. In insectivores, carnivores, monkeys and man the ground substance, as a rule, takes on a reddish tinge with cresyl violet, but in the rodent and the bat this is not so constant or so marked. The ground substance of the medial nucleus always stains the deepest, and that of the anterior, basal and intermediate nuclei less deeply. This fact can be observed from the photograph where the ground substance of the medial nuclear group appears a uniform grey.

Investigations regarding the function and connexions of the whole medial group of nuclei in rodents (for instance in the rabbit which is commonly used for laboratory purposes) do not allow reliable conclusions to be inferred regarding the fibre connexions of this nuclear group in man, because, in the rabbit, this group consists mostly of those nuclei which are only feebly developed in man. On the other hand, it may be assumed that the medial nuclear group of the dog, and particularly that of the monkey, subserves the same functions, and therefore has similar fibre connexions, as in man.

(*b*) *The lateral group of nuclei*

This consists of a single nucleus, the lateral mamillary nucleus. Throughout the mammalian scale this shows a typical shape and position. It is always situated lateral to the medial group, and ventro-lateral to the fornix. Its shape is generally oval or round, and its cells are always the largest and most deeply staining of the whole mamillary body. In the series of animals investigated it shows neither a complete regression nor a differentiation.

In bats and rodents it is very clearly formed, and in the rabbit it produces a macroscopically visible swelling in the hypothalamus.

In insectivores it is remarkably indistinct; in the hedgehog it is present no doubt, though feebly developed, whilst in the mole it cannot be defined as a distinct nucleus. As I have already mentioned, when describing the mamillary body of the mole, I consider that the scattered nerve cells found ventro-lateral to the fornix show distinct characteristics which allow them to be considered as the homologue of the lateral nucleus.

In the carnivores the lateral nucleus is very well developed, though in the dog it can only be followed for a short distance. In the monkey its shape and position are typical. In man it is clearly developed but is divided into two bands. From its typical position near the fornix it can be inferred that it is closely associated with that structure, confirmation being found in the fact that it generally appears when the fornix can no longer be traced as a distinct bundle (at least in Nissl preparations). Its small size, however, makes it most improbable that it receives all or even the majority of the fibres of the fornix. In fact, degeneration experiments have proved the lateral nucleus to be independent at least of the majority of the fibres of the fornix.

Within the whole mammalian scale the elements included under the term lateral mamillary nucleus are no doubt homologous, and the function of the nucleus can be assumed to be the same in all mammals. The shape, size, density and cell processes of the nerve cells of this nucleus are prone to great variations in the animals investigated.

For experimental operations on the lateral mamillary nucleus, the rodents, and especially the rabbit, seem to be the most suitable. The lateral nucleus of the rabbit is particularly large, sharply demarcated, and relatively distant from the other nuclei.

(*c*) *The dorsal group of nuclei*

The supramamillary and perimamillary nuclei comprise this group and show a close topographical relationship to the mamillary body. It cannot be stated with certainty how far they are functionally related to it. In all mammals (including man) the dorsal nuclear group can easily be defined, but the development of the component nuclei varies.

In the bat, the supramamillary nucleus and the perimamillary nucleus are both very well developed, and this dorsal nuclear group is particularly well differentiated in the insectivores. Both these nuclei in the hedgehog and the mole are large and broad. In rodents, the supramamillary nucleus is well developed, but the perimamillary is only poorly so, being best developed in the mouse, poorly in the squirrel and poorest in the rabbit.

In the carnivores also, the dorsal nuclear group is well developed, more so in the polecat than in the dog. The monkey shows a moderately developed supramamillary nucleus and an easily discernible perimamillary nucleus. In man the supramamillary nucleus can readily be seen, but only extends for a short distance, whilst the perimamillary nucleus is very feebly developed. In dog, monkey and man this group is interrupted by the thalamo-mamillary tract.

The fact that I found the supramamillary nucleus present in man contradicts the statement of Grünthal (1933) that the absence of the supramamillary nucleus should be considered a human feature.

(*d*) *The volume of the nuclei of the mamillary body*

Examination of the mamillary body from a phylogenetic point of view has shown that some nuclei of the lower mammals are only feebly developed in the higher mammals. Thus the question arises whether the mamillary body, as a whole, has undergone a reduction in the course of evolution, or whether single nuclei increase or, at least, do not diminish in size whilst others are reduced. A superficial examination of this series seems already to favour the second assumption. In the higher mammals the medial nucleus is so large that it is probably more correct to speak of a progressive rather than a regressive development.

This question, however, can be answered accurately only by determining

the volume of the single nuclei, and it is of special interest in so far as Grünthal maintains that the hypothalamus is subjected to a regressive development in the ascending animal scale. This view Le Gros Clark (1936) does not share, pointing to the well-developed medial nuclei in man.

The volume of the three nuclear groups, medial, lateral and dorsal, whose equivalence in the different mammals seemed to me beyond doubt, has been measured separately, and compared with the volume of the thalamus and hypothalamus.

The thalamus is taken to consist of (*a*) the epithalamus (ganglia habenulae, taenia thalami),[1] (*b*) the metathalamus (corpus geniculatum mediale and laterale), and (*c*) the thalamus proper or dorsal thalamus (prethalamic,[2] anterior, lateral, medial, ventral, reticular, central, posterior and prebigeminal nuclear complex (M. Rose)). According to M. Rose the following purely topographical nuclear complexes are implied when speaking of the hypothalamus: dorsal, medial, lateral, ventral, suprapeduncular and mamillary. The substantia nigra has not been included.

The boundary between the thalamus and hypothalamus is not always sharply defined, but the doubtful zone is relatively narrow and occurs infrequently.

Regarding the demarcation of the three nuclear groups of the mamillary body, the sharp outline of the dorsal nuclear group is often artificial whilst the lateral group in insectivores, particularly in the mole, can only be determined approximately.

The volume of the nuclei on each side has been ascertained, and in all animals the volume of the ventricle has also been determined.[3]

Table I

	Volume of the hypothalamus in c.mm.	Volume of the thalamus in c.mm.	Ratio: $\frac{\text{hypothalamus}}{\text{thalamus}}$
Bat	5·0	6·3	1:1·3
Hedgehog	51·2	61·4	1:1·2
Mole	12·4	23·5	1:1·9
Mouse	10·2	14·1	1:1·4
Rabbit	71·3	176·1	1:2·5
Squirrel	84·3	192·2	1:2·3
Polecat	66·0	192·3	1:2·9
Dog	188·7	703·7*	1:3·7
Monkey	273·5	880·0	1:3·2
Man (3 months old)	913·5	4590·0	1:5·0

* The thalamic volume of the dog is a little greater than here indicated because, owing to the technical difficulties, the most posterior parts of the thalamic nuclei could not be reconstructed.

From Table I it can readily be seen that in the ascending animal scale the volume of the thalamus shows a regular increase compared with that of the

[1] The epiphysis has not been included.

[2] Except in the thalamic volume of dog and man.

[3] Embryological studies on the rabbit have since convinced me that the prethalamic nuclear complex does not belong to the thalamus but to the hypothalamus. This nuclear group, however, being rather small, this statement is of no particular importance for the total volume of the parts of the brain measured here.

hypothalamus. Whereas in the bat the ratio of the thalamic volume to that of the hypothalamus is only 1·3, it amounts to 2·5 in rodents, 3·7 in carnivores and as much as 5·0 in man.

Table II

	Volume of the total grey matter of the mamillary body in c.mm.	Ratio: $\frac{\text{mamillary body}}{\text{hypothalamus}}$
Bat	0·23	0·046:1
Hedgehog	1·62	0·032:1
Mole	1·92	0·155:1
Mouse	0·52	0·051:1
Rabbit	5·01	0·070:1
Squirrel	3·22	0·038:1
Polecat	4·99	0·076:1
Dog	15·67	0·083:1
Monkey	7·97	0·029:1
Man (3 months old)	38·02	0·042:1

From Table II it can be seen that the ratio of the volume of the mamillary body to that of the hypothalamus shows rather marked variations. Thus in the mole the relative volume of the mamillary body is particularly great. Similarly, the rabbit and carnivores show a relatively large mamillary body. It may also be pointed out that in both monkey and man the ratio is relatively small, and in view of the fact that the mamillary body in both is well developed one is led to suppose that there has been no relative decrease in the grey matter of the hypothalamus. Man shows about the same ratio as the bat and squirrel, the smallest ratio of the investigated animals being in the monkey.

Table III. *Volume of the nuclear groups as percentage of volume of whole mamillary body*

	Dorsal	Medial	Lateral
Bat	47·8	47·8	4·4
Hedgehog	51·9	42·5	5·6
Mole	40·6	54·8	4·6
Mouse	26·9	67·3	5·8
Rabbit	23·1	65·3	11·6
Squirrel	26·4	70·2	3·4
Polecat	30·7	64·7	4·6
Dog	24·0	70·8	5·2
Monkey	39·1	54·3	6·6
Man (3 months old)	13·3	80·6	6·1

From Table III it can be seen that in almost all mammals the lateral nuclear group has an approximate constant value, the variations in volume relative to the whole mamillary body being rather small in all except the rabbit. This suggests that functionally the lateral nucleus is intimately related to the rest of the grey matter of the mamillary body, otherwise this constant ratio is difficult to understand. Topographically, too, this connexion can be recognized in rodents and in the bat. In monkey and man, where the medial nuclear group is separated by a medullary capsule from the lateral nucleus, the connexion is less conspicuous.

In addition, from Table III it can be seen that as one ascends the animal

scale the volume of the medial nuclear group increases at the expense of the dorsal group. In the bat and insectivores the volume of these two groups is equal, whereas in the hedgehog the dorsal group has an even greater volume than the medial. In rodents and in the polecat the volume of the medial nuclear group amounts to more than twice that of the dorsal, whilst in the dog it is almost three and in man six times the size. In the monkey, however, it amounts to only one and a half. Thus the medial nuclear group not only shows a marked increase as a whole, but this increase is exclusively due to the increase of the medial nucleus. The basal and intermediate nuclei, which in the bat, insectivores and rodents are large and can be estimated to amount to about one-half of the total volume of the medial group, form only a fraction of the volume in the dog, monkey and man.

The comparison of the volume of the different nuclear groups of the mamillary body with that of the thalamus or hypothalamus is given in Tables IV and V.

Table IV. *Volume of the lateral group of nuclei (percentage of the hypothalamic volume)*

Bat	0·20	Squirrel	0·13
Hedgehog	0·17	Polecat	0·34
Mole	0·72	Dog	0·43
Mouse	0·28	Monkey	0·19
Rabbit	0·81	Man (3 months old)	0·25

From Table IV it may be seen that the lateral nuclear group does not show any great variations except in the mole and the rabbit. It has already been shown (Table II) that the mole has a large mamillary body, whilst its lateral nucleus is of average relative volume (Table III). The rabbit, on the other hand, combines an average-sized mamillary body with a relatively large lateral nucleus. As a rule, the lateral nuclear group seems to have a fairly constant volume not only relative to the mamillary body but also to the entire hypothalamus. This would tend to favour the assumption that the lateral nuclear group has no connexions with the thalamus (or, at least, not with those parts that show a progressive development in higher mammals).

With the medial nuclear group the relationship is different, owing to its connexions with the thalamus, and one assumes that there is a definite ratio between the volumes of the grey matter of these two structures.

Table V. *Volume of the dorsal and medial nuclear groups (percentage of the thalamic volume)*

	Medial	Dorsal
Bat	1·70	1·70
Hedgehog	1·11	1·40
Mole	4·50	3·30
Mouse	2·48	0·98
Rabbit	1·86	0·65
Squirrel	1·17	0·44
Polecat	1·67	0·79
Dog	1·57	0·53
Monkey	0·49	0·35
Man (3 months old)	0·68	0·10

From Table V it is seen that in spite of a relative increase in the ratio of the volume of the thalamus to that of the hypothalamus as shown in Table I, the volume of the medial nuclear group, expressed as a percentage value of the volume of the thalamus, differs only slightly in the mammals, apart from monkey and man, and also the mole. That is, the values show no decline until the primates are reached. But even in man this value is only 2·5 times smaller than in the bat.

In man, however, the thalamus is five times the volume of the hypothalamus, whereas in the bat it is only 1·3. Furthermore, in the hedgehog the percentage value of the medial group is not twice that of man; its thalamus, however, is only 1·2 times the size of its hypothalamus. The dog and polecat, in which the thalamus is about three times the volume of the hypothalamus, show the same (or even higher) percentage values for the medial nuclei in comparison with the bat and hedgehog, whose thalamus:hypothalamus ratio is 1·2. The monkey shows a great relative decrease in the percentage value of the medial nuclear group.

As a rule, however, it may be assumed that the volume of the medial nuclear group increases as the thalamus develops, although this increase, as is to be expected, does not run strictly parallel with the phylogenetic increase in volume of the thalamus as a whole. This statement brings out another fact. One can speak of the medial nuclear group as probably showing a progressive development in man and higher mammals; it is certainly not reduced.

Similarly, it may be inferred that the lateral nuclear group is probably not reduced, as it shows a constant relationship to the mamillary body (see Table III).

Unlike the medial nuclear group, the figures in Table V show clearly that the percentage value of the volume of the dorsal nuclear group, calculated in relation to the thalamic grey matter, shows a decrease rather in excess of what might be expected in view of the relative increase of the thalamus. This suggests that the dorsal nuclear group has no connexions with the thalamus.

In man it seems to have undergone a distinct reduction. It cannot be seen with sufficient certainty from the percentage values whether it is also reduced in the higher mammals generally. In rodents and carnivores, however, the dorsal nuclear group appears to have been reduced.

SUMMARY

In all mammals the mamillary body is well developed. Three groups of nuclei can be distinguished: medial, lateral and dorsal.

The medial group consists of nuclei of which some are better developed in the lower mammals, others in the higher.

In the higher mammals the medial nucleus, probably equivalent to the medial and anterior nuclei of the lower mammals, is better developed, whereas the basal and intermediate nuclei show a poorer development.

In the higher mammals the volume of the medial nuclear group, as a whole, shows a relative increase in association with that of the thalamus, a relationship that becomes obvious by determining their respective volumes.

The lateral group of nuclei in all mammals bears a fairly constant relation to the volume of the mamillary body as a whole, as well as to the whole hypothalamus. The lateral nuclear group seems to have no connexion with the thalamus.

The dorsal nuclear group, very well developed in lower mammals, undergoes a reduction in man and probably in the other higher mammals as well, whilst its volume decreases relative to that of the mamillary body, hypothalamus and thalamus. It is probably not connected with the thalamus.

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ABBREVIATIONS IN THE FIGURES

cs, corpus subthalamicum; *F*, fornix; *i*, n. infundibularis; *ma*, n. mamillaris anterior; *mam-inf.* n. mamillo-infundibularis; *mb*, n. mamillaris basalis; *mc*, n. mamillaris centralis; *mi*, n. mamillaris intermedius; *ml*, n. mamillaris lateralis; *mm*, n. mamillaris medialis; *perim*, n. perimamillaris; *pl*, n. hypothalami posterior lateralis; *pp*, pes pedunculi; *ppl*, n. hypothalami posterior post-lateralis; *pre*, n. praemamillaris; *psi*, n. medialis postremus hypothalami; *sma*, n. supramamillaris; *sp*, suprapeduncular nuclear complex; *VA*, bundle of Vicq d'Azyr.

ANASTOMOSES BETWEEN THE RECTAL AND UTERINE VEINS FORMING A CONNEXION BETWEEN THE SOMATIC AND PORTAL VENOUS SYSTEM IN THE RECTO-UTERINE POUCH

By E. G. WERMUTH

The existence of anastomoses between the somatic and portal venous systems in the region of the rectum has been known for a long time. The question as to whether there are anastomoses between the rectal and uterine veins above the levator ani muscle was raised, first, by clinical and pathological observations in cases of pylephlebitis of the liver due to purulent endo- and parametritis, and secondly, by the presence of a white discharge from the uterus in some cases of congestion of the portal system.

Before describing my own investigations it may be useful shortly to review the literature dealing with the veins in the female pelvis in general and particularly that dealing with the venous connexions between the genital tract and the rectum. Rauber-Kopsch, Sieglbauer, Spalteholz and Tandler recognize the following venous plexuses in the pelvis, named according to the organs on which they lie: pudendal plexus (in front of the bladder), vesico-vaginal plexus, utero-vaginal plexus, haemorrhoidal plexus. The first three plexuses are in wide communication and form a unit from which the haemorrhoidal plexus is distinguished because it flows to the portal vein and has no wide connexion with the other pelvic plexuses (see below under Peham-Amreich). Further details about the veins of the rectum and uterus are given by Poirier-Charpy.

The main flow of uterine blood goes to the hypogastric vein through the utero-vaginal plexus and also to the ovarian vein which is a direct cranial continuation of the utero-vaginal plexus. There is another path caudally to the internal pudendal vein through the pudendo-labial plexus, and there is a smaller path to the veins of the lower abdominal wall by means of the round ligament of the uterus.

The veins of the rectum as far as the anus flow into the portal vein through the superior haemorrhoidal vein. The latter originates in the venous plexus of the rectal columns of Morgagni, from which come two main veins (left and right) running in the submucous tissue of the rectum for 5–6 cm., then breaking through the muscular wall in order to form the external haemorrhoidal plexus which lies under the serosa of the ampulla recti on both sides. They then end in a vein on each side, joining with the superior haemorrhoidal vein. The

anastomoses of the above venous region are described by Poirier-Charpy as follows:

(1) The inferior haemorrhoidal veins. These are very small vessels in the subcutaneous tissue around the lower margin of the external anal sphincter muscle. They receive some blood from the venous plexus in the rectal columns and passing through the ischio-rectal fossa establish connexions with the internal pudendal vein.

(2) Anastomoses laterales. These are several veins arising from the rectal wall 5–6 cm. above the anus. Together with some vaginal veins they form a so-called intermediate haemorrhoidal vein on each side, flowing into the hypogastric vein.

The name and significance of the intermediate haemorrhoidal vein vary greatly throughout the literature. Authors using the Jena nomenclature do not mention this name nor an analogous vein. Other authors show remarkable differences in the description of the intermediate haemorrhoidal vein. Corning calls it a paired vessel breaking through the rectal wall in the sphincter region without relation to the genital veins in its further course. The original picture Corning is referring to (which is also used by Rauber-Kopsch) is given by Nuhn and shows "two veins cut off just before they join the hypogastric vein and collecting all veins from the pelvic plexus". There is a certain resemblance to Poirier-Charpy's intermediate haemorrhoidal vein, so far as the latter also collects the blood from the rectum and the genital organs. According to Nuhn, however, it appears that the entire rectal blood flows to this vein and therefore to the hypogastric vein, but this is denied by all the other authors. They all agree that the main flow of the rectal blood goes to the portal vein. Peham-Amreich says that all veins of the female urogenital tract are in wide connexion with those of the rectum and form practically a single large plexus. For these reasons the ligature of the main veins leading to the hypogastric vein does not always prevent the spread of septic thrombophlebitis, because the way to the portal vein still remains open. Although the pictures of Peham-Amreich show much detail, they do not show any path to the portal vein as mentioned above, but the pararectal space where the respective anastomoses should be expected is shown as containing no anastomoses at all. Kownatzki gives a very detailed description of the female pelvic veins, but does not mention an intermediate haemorrhoidal vein or any corresponding vein. From the literature, therefore, it appears that the relations between the rectal and genital veins are not certain and the statements of the various authors contradictory. Further, it seems that a wide communication between the external haemorrhoidal plexus and the utero-vaginal plexus, similar to the connexion between the latter and the vesical plexus, is usually denied.

The object of these investigations was to discover the exact relations of the venous anastomoses between the genital and rectal plexuses, and particularly to seek for evidence of direct venous connexions between the rectum and the uterus.

MATERIAL AND TECHNIQUE

The results of investigating blood vessels in the cadaver by injection methods do not necessarily reflect the circulatory conditions in the living subject, for although a vessel may fill with the injection material it does not follow that in the living the blood would fill the vessels to a similar degree under physiological conditions. An example of this can be seen in investigations on the rami communicantes in the circle of Willis, and the difficulties are even greater in the case of the veins where, to reproduce in the cadaver all the conditions on which venous flow depends, is almost impossible, especially as slight changes may result in pathological effects in the living. This must be borne in mind in interpreting the results of venous injections in the cadaver. It is possible, however, to draw conclusions as to the state of affairs in the living if several specimens are injected from different directions and give consistent results.

The investigation was carried out on fourteen bodies a few hours after death. In twelve cases a retrograde injection through the superior haemorrhoidal vein was used. The pelvis as a whole was separated from the body and a cannula inserted into the superior haemorrhoidal vein as it lay in the mesentery of the sigmoid colon. Warm water was first run into the cannula, and then the injection material by means of high pressure syringes operated by screwing. The filling of the internal haemorrhoidal plexus was watched by a rectal speculum. As soon as this plexus became filled and a constant pressure felt upon screwing the syringe, the injection was considered sufficient. In two cases an injection was made in the natural direction through the dorsal vein of the clitoris. In these cases the amount of material injected was judged entirely by the pressure in the syringe. In some cases the hypogastric veins on one or both sides were also injected.

If anatomical preparations are required, the most suitable material for injection is "Revertex", which is a rubber derivative in the form of a thick fluid which remains elastic. Stained with cinnabar it gave a good shadow with X-rays. In some cases specimens were cleared by Spalteholz's method, and in these cases the injection material consisted of Teichmann's injection material or a mixture of water-glass and kaolin. The latter took much less time to prepare. In addition, Tandler's cold fluid gelatine was tried, stained with ultramarine blue; this material however, although sufficiently fluid to fill capillaries, was not suitable for macroscopic specimens on account of its small elasticity.

After injection the tissue was preserved by the injection of 2 % formalin into the arteries. The "Revertex" specimens were transferred to 70 % alcohol, which causes the pelvic connective tissue to remain much softer than does formalin. The Teichmann specimens were kept in 2 % formalin, and the kaolin specimens in absolute alcohol before clearing by the Spalteholz method. The clearing process should begin by the removal of pigment with peroxide of hydrogen, and for this purpose it is of advantage to remove the pelvic viscera

from the pelvis. After dehydration in ascending concentrations of alcohol the specimens are kept permanently in a special solution of chaulmoogra oil and benzyl benzoate, which renders the entire specimen more or less transparent. It is of advantage to have the specimen as thin as possible. In spite of every care in preservation it was not possible to avoid the presence of some gas bubbles.

RESULTS OF THE INVESTIGATIONS

Four typical cases are described, the findings in the other cases being very similar. All anastomoses between the rectal veins and tributaries of the hypogastric vein are in a space which may be referred to as the pararectal space. This space is filled with connective tissue ("tela urogenitalis" of Tandler) and is bounded medially and caudally by the rectum, laterally by the hypogastric and uterine veins, cranially by the recto-uterine ligament, and medially and cranially by the peritoneum of the pouch of Douglas.

The veins are described according to the direction of injection and not according to the direction of blood flow in them. In the descriptions of the veins, in some cases the calibre is indicated by accompanying measurements. Cases 1–4 were injected through the superior haemorrhoidal vein. Cases 5 and 6 through the dorsal vein of the clitoris.

Case 1 (Fig. 1)

The superior haemorrhoidal vein lies in the pelvic mesocolon and comes into relation with the upper part of the rectum on its left posterior wall. There it divides into a right and left main branch (5 mm. in diameter). The right branch reaches the right side of the rectum by going around the posterior wall at the level of the second sacral vertebra. Each of the main branches divides into a lateral branch (*A*, 5 mm.), and a medial branch (*B*, 3 mm.), approximately at the level where the peritoneum is reflected from the rectum to the posterior fornix of the vagina. The lateral branches on both sides continue the direction of the main branch by running along the rectum. On the right side the medial branch (*B*) divides into numerous small branches penetrating the rectal wall. On the left side the medial branch divides into two branches, medial and lateral. The former continues the direction of the main medial branch (*B*) while the latter continues laterally and caudally to communicate with the main lateral branch (*A*). In spite of the names given in the literature, the superior haemorrhoidal vein does not form a plexus comparable with other pelvic venous plexuses. The superior haemorrhoidal vein communicates with the hypogastric vein, the uterine veins, and the utero-vaginal plexus by the following anastomoses which are described in a cranio-caudal direction.

Right side.

(1) A vein (1 mm. in diameter) runs cranially after arising from the main branch at the level of the second sacral vertebra, and enters the hypogastric vein at the arcuate line.

(2) A vein (1 mm.) arises from *B* and runs laterally. After receiving numerous small subserous veins, it enters the hypogastric vein 2 cm. more peripherally than (1).

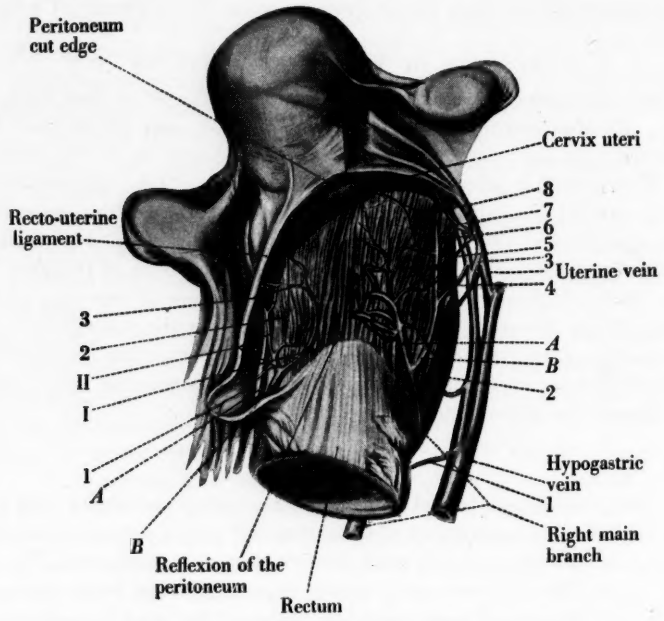

Fig. 1. Peritoneum of the pouch of Douglas removed, displaying the cervix and the right side of the body of the uterus, as well as the retroperitoneal portion of the rectum. Recto-uterine ligament preserved on the left side and drawn dorsally. On the right side the pararectal space is widely opened.

Left side:

A, *B*, I, II, branches of the superior haemorrhoidal vein.

1, anastomotic vein disappearing in the recto-uterine ligament.

2, 3, anastomotic veins passing dorso-ventrally to the uterus.

Right side:

A, *B*, branches of the superior haemorrhoidal vein.

1–7, anastomoses between the rectal veins and branches of the hypogastric vein passing laterally and cranially (not figured on the left side).

8, anastomotic vein passing dorso-ventrally to the uterus.

(3) Another vein (1 mm.) comes directly from the posterior wall of the rectum and not from any visible branch of the superior haemorrhoidal vein. Passing cranially it reaches the uterine vein just before the latter joins the hypogastric vein.

(4) A vein (1 mm.) coming from *A*, enters the hypogastric vein 1 cm. peripherally from the junction with the uterine vein.

(5) A vein (1 mm.) is formed by the junction of two equal tributaries arising from *A* and *B*. It splits into two short branches one of which enters the uterine vein a little peripheral to the junction of (3) with the uterine vein. The other branch enters a vessel which lies caudal to the uterine vein and which runs parallel to it towards the lateral border of the uterus.

(6, 7) Two 1 mm. veins arising from *A*. They also enter the vein mentioned above which runs parallel with the uterine vein.

(8) A 1 mm. vein formed by the junction of two equal tributaries which arise from *A* and *B*. It passes dorso-ventrally and reaches the utero-vaginal plexus on the lateral margin of the uterus.

Left side.

(1) A vein (0·5 mm.) arises from II and breaks up into a fine network which disappears in peritoneal tissue of the recto-uterine fold.

(2) A vein, 1 mm. in diameter and 2·5 cm. in length, arises from II. It passes directly ventrally and a little laterally to the lower end of the lateral margin of the uterus, there entering the utero-vaginal plexus.

(3) Two small veins (0·5 mm.) coming from both I and II, join and form a vein, which passes cranially and disappears in the recto-uterine fold, where the injection stops.

Case 2 (Fig. 2)

The branches are similar to case 1 with the exception that here the left medial branch (*B*) divides into three branches; a lateral branch (I), an intermediate (II) and a medial (III). This division takes place on the lateral rectal wall about 2 cm. caudal to the peritoneal reflexion. The following anastomoses were found:

Right side.

(1) A branch, 3 cm. long and 1 mm. in diameter, arises from *B*. It runs dorso-ventrally and enters the lateral part of the posterior vaginal fornix. Just before entering the fornix it gives a fine branch which runs medially to the anterior rectal wall and enters it.

Left side.

(1) There is a vein, 1 mm. in diameter and 2 cm. long, running directly dorso-ventrally and entering the lower part of the lateral margin of the uterus. This vein is formed by the junction of two veins. One of them arises from II just behind the point of division of *B*, the other from the branches formed by the breaking up of III.

(2) There is a short vein (1 mm.) passing laterally and cranially to the utero-vaginal plexus. It arises from I, 3 cm. caudal of the point of division of *B*.

Further anastomoses were found on both sides leading laterally and cranially to the veins on the pelvic wall. The arrangement was very similar to

veins 1–4, described on the right side of case 1, and are not described because the main stress is laid on anastomoses which pass directly to the uterus.

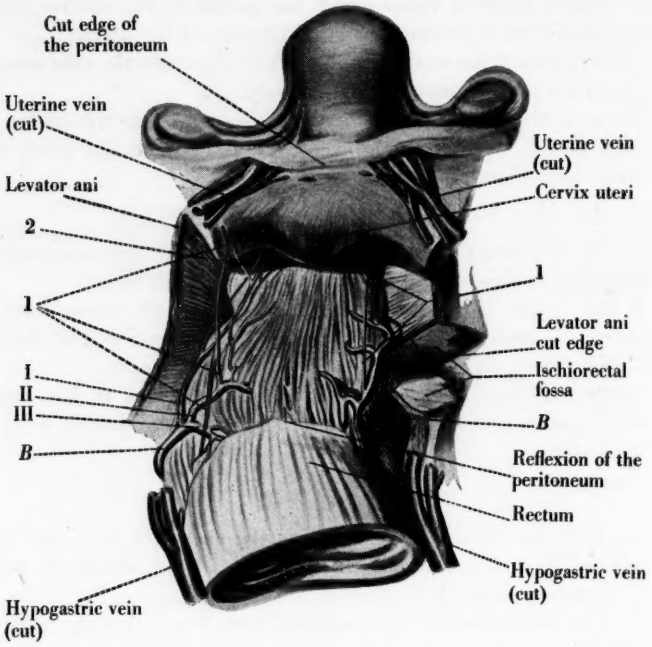

Fig. 2. Peritoneum of the pouch of Douglas removed on both sides. Uterine and hypogastric veins cut on both sides. Rectum and uterus drawn apart. On the right side coronal section through the levator ani and ischiorectal fossa (latero-cranial anastomoses between rectal and hypogastric vein not figured).

Left side:

B, I, II, III, branches of the superior haemorrhoidal vein.

1, 2, anastomotic veins passing dorso-ventrally to the uterus.

Right side:

B, branch of the superior haemorrhoidal vein (further branches not described).

1, anastomotic vein passing dorso-ventrally to the uterus.

Case 3

The situation and branches of the main veins are similar to the previous cases. Anastomoses are as follows:

Right side.

No anastomoses from the external branches of the superior haemorrhoidal veins. However, there were found two anastomoses coming from remarkable venous arches. These arches are formed by veins (1 mm.) which leave the

rectal wall, form a subperitoneal arch, giving an anastomotic branch from their convexity and returning to the rectal wall near where they arose.

(1) Such a venous arch leaves and re-enters the rectal wall at the level of the third sacral vertebra. It gives a branch, 1 mm. in diameter and 2 cm. long, which passes dorso-ventrally and reaches the utero-vaginal plexus 0·5 cm. laterally to the lower end of the lateral margin of the uterus.

(2) A similar vein which enters the lateral margin of the uterus; it is shorter than (1) because it arises from a venous arch 2 cm. caudal to the first arch.

Left side.

(1) A vein, 1 mm. in diameter and 3 cm. in length, comes from a short medially directed branch of *B*. It passes dorso-ventrally to the lower end of the lateral margin of the uterus.

Case 4

Here the hypogastric vein was injected as well as the superior haemorrhoidal vein. The arrangement of the main branches is similar to the previous cases, but the external branches make no anastomosis with the uterus. There is only one anastomosis arising directly from the rectal wall. A vein, 3 mm. in diameter and 2 cm. in length, emerges from the anterior rectal wall near the midline where the wall has no external veins. This is approximately 5–6 cm. above the anus. The vein passes in a dorso-ventral direction to the lower end of the left lateral margin of the uterus.

Cases 5 *and* 6

These cases were injected through the dorsal vein of the clitoris. The urogenital venous plexus and the parietal veins were found to be completely filled with the injection material, but none of the rectal veins was filled—neither the external nor the internal plexus, the latter being watched by a speculum. Further, no injected anastomoses were found comparable to those described in the previous cases.

Observations using the Spalteholz method

Specimens rendered transparent by this technique confirmed the results of anatomical dissection so far as the anastomoses passing to the uterus were concerned. Most of the cases resembled the findings in cases 1 and 2. Anastomoses between the rectal veins and the veins of the pelvic wall (such as case 1, right side 1–4) could not be investigated because the organs had to be removed from the pelvis as a whole after injection through the superior haemorrhoidal vein.

DISCUSSION

As a fairly constant venous arrangement was found, the evidence from the above cases enables the following schematic summary to be made of the branches of the superior haemorrhoidal vein and its anastomoses. In the first

place the name "external haemorrhoidal plexus" does not appear to be very suitable, for on the rectum the branches of the superior haemorrhoidal vein were distinct veins not differing greatly from those in other parts of the intestine. This distinguishes the so-called external haemorrhoidal plexus from the other pelvic venous plexuses. In the latter case, the name plexus is justified for the veins constituting them are wide blood-spaces anastomosing frequently and widely. For the lack of any other name, "external haemorrhoidal plexus" is retained in this paper

The scheme of the branches is as follows. In the midline the anterior rectal wall is free from macroscopic veins, as the veins on both sides do not show any evident anastomoses on the external surface. The superior haemorrhoidal vein gives rise to a right and left main branch. The right branch passes behind the rectum to the right side. Then on each side the right and left main branch divides into a lateral (*A*) and medial branch (*B*), where the peritoneum is reflected from the rectum to the posterior vaginal fornix. Subsequent divisions vary slightly in different cases. In these divisions the veins do not diminish much in calibre and do not subdivide very greatly, but continue their course almost vertically downwards and all pierce the muscular wall of the rectum about 5–6 cm. above the anus. They then run in the submucous tissue.

In the anastomoses between the somatic and portal venous systems in the pelvis two groups can be recognized:

(*a*) Latero-cranial anastomoses. Veins passing laterally and cranially from the rectum to the hypogastric vein and its branches as described on the right side of case 1. There are usually four or five anastomoses on each side. They arise from branches *A* or *B*, but more frequently from the lateral branch *A*.

(*b*) Dorso-ventral anastomoses. Veins passing dorso-ventrally which arise more caudally than the first group. There are one or two anastomoses on each side consisting of veins 2–3 cm. in length and 1 mm. in diameter arising from the rectum and running in the connective tissue of the recto-uterine ligaments (pararectal space) towards the lower end of the lateral margin of the uterus. Their origin from the rectum was fairly constant in seven cases. They arose from the tributaries of the main branches, more often from branch *B*. In case 3 two veins were found arising directly from the rectal wall apart from tributaries of the main branch. These showed remarkable venous arches. In case 4 there was only one, but a much bigger, anastomosis arising directly from the anterior rectal wall.

These anatomical facts permit the discussion of the following questions:

(*a*) What is the significance of these anastomoses in the normal and pathological blood flow in the female pelvis?

(*b*) What is the relationship of these anastomoses to the well-known circumanal anastomoses?

Owing to the disadvantages of the injection methods already mentioned, a complete answer to these questions cannot be given here. Of the first question it may be said that the venous return from pelvic organs is extremely variable.

It is influenced by physiological filling of the rectum and bladder, pregnancy, and pathological changes such as tumour formation, inflammation, etc. These produce greater changes in the blood flow of the pelvis than similar conditions in the abdomen. Pelvic blood flow is affected more than abdominal blood flow in such circumstances because of the rigidity of the pelvic walls.

The wide pelvic venous plexuses are to some extent able to prevent obstruction to the venous return, as compression of a plexus is more difficult than of a single vein, and also the wide and frequent anastomoses of a plexus would allow the blood to find some alternative route. On the other hand, plexuses have the disadvantage that they slow the blood flow because of their large total diameter, thereby further decreasing the speed of flow which is already low due to the small pressure difference between peripheral veins and the more central trunks. Plexuses are found in relation to the urogenital system, but these drain into the inferior vena cava where the difference in pressure from that in the peripheral veins is sufficient to overcome the slowing action of the plexuses. The rectal veins, on the other hand, represent the most peripheral portion of the portal system which has no valves, and where the pressure differences are much less than in the caval system. For this reason, in the rectum the further slowing of the blood flow by a venous plexus has been avoided.

Cases 5 and 6, which were injected in the direction of normal blood flow, give evidence that blood does not flow into the rectal veins from the urogenital organs as long as conditions are normal. Although the anastomoses described form a possible route from the uterus to the rectum, normally the blood flow in them is negligible. This might be due to valves in the anastomotic veins, or to their small size compared with the veins of the urogenital plexus. The anastomotic veins might become important, however, if flow in the caval system were impeded by any cause. Furthermore, infection might spread along these anastomotic veins in spite of their negligible blood flow.

On the other hand these anastomoses might become functional in general portal congestion (cirrhosis of the liver, thrombosis of the portal vein, tumours, etc.), or in local obstructions of the venous outflow from the rectum. These congestions were imitated by injection of the superior haemorrhoidal vein, when the recto-uterine venous anastomoses were demonstrated.

In discussing the second question, it is known that the degree of haemorrhoid formation does not correspond to the degree of portal obstruction in cirrhosis of the liver. This may be accounted for by a large formation of varicosities around the cardia of the stomach which act as the main path for relief of the congested portal system. There is no doubt, however, that rectal anastomoses also play a part in the relief of portal congestion, but it seems that this relief is mainly provided by intrapelvic venous anastomoses, rather than by the circumanal. This is suggested by those cases where the superior haemorrhoidal vein was injected, thus imitating artificially the conditions of portal congestion. In these cases the intrapelvic anastomoses as well as the

internal haemorrhoidal plexus were well filled, but no injection could be found in the circumanal anastomoses in the ischiorectal fossa.

The final confirmation of the importance of the intrapelvic venous anastomoses can only come from investigations on cases of established portal congestion. As a rough means of investigation at autopsy a useful method would be to examine the recto-uterine pouch for enlarged veins, and also to examine the veins of the pararectal space. However, an accurate investigation is only possible by the use of some injection and dissection technique.

SUMMARY

1. A description is given of the venous anastomoses between the uterus and the rectum, based on the findings in several normal pelves. These constitute previously undescribed anastomoses between the somatic and portal venous systems.

2. In normal and in pathological conditions the significance of these anastomoses is discussed in the pelvic blood flow, and in the spread of infection from the uterus and parametrium towards the portal vein.

3. In portal congestion it is suggested that these anastomoses are of more importance than the circumanal anastomoses.

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ANATOMICAL NOTES

A MODIFICATION OF THE TECHNIQUE OF WAX PLATE CONSTRUCTION

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Following the classical method of wax plate reconstruction devised by Born, the image of the histological preparation is projected downwards by means of a vertically disposed optical system on to a sheet of paper. It is viewed by reflection from the surface of the paper and is then traced in pencil by hand. The paper is incorporated with a wax plate, the thickness of which is arranged to correspond to the linear magnification of the image, and the enlarged reproduction of the histological section is finally cut out with a sharp knife. This procedure has been modified in the following way:

The image is projected by means of a horizontal photomicrographic apparatus of conventional design on to a plate glass screen. This is rigidly supported upon a

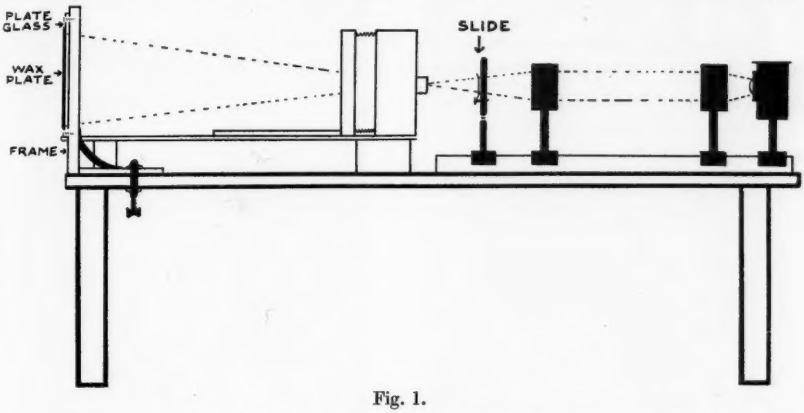

Fig. 1.

sliding wooden frame-work. On the back of the plate glass a wax plate is laid and retained in position by means of spring clips. The plates may be obtained ready made to any specified dimensions from the Dental Manufacturing Company, and at a cost below that of the raw material required for their manufacture in the laboratory.

The image is focused upon the surface of the wax which is in contact with the plate glass, the wax thus acting as a ground glass surface. The optical arrangements are shown diagrammatically in the accompanying figure. The optical qualities of the image are closely comparable to those upon a fine-grain ground glass screen.

The cutting out process is carried out directly upon the wax plate, the image being viewed throughout by transmitted light. In order to avoid dazzle all excised pieces of wax are left *in situ* until the cutting out is complete. The advantages of the procedure described are as follows:

1. Use is made of a photomicrographic bench of conventional type. Such an apparatus is likely to be part of the standard equipment of any laboratory, and further to comprise the best optical system available therein.

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The necessity for an additional and in any case less perfect optical system is thus obviated.

2. The considerable labour and laboratory space required for the preparation of the wax plates is avoided by the use of ready-made plates. This involves no increase of cost.

3. The two operations of tracing and cutting out are reduced to one by direct cutting out of the image projected upon the wax plate. This reduces greatly expenditure of time, and increases accuracy.

4. Accuracy of working is greatly favoured by the optical and mechanical conditions which result from the use of transmitted light and of a rigidly supported screen of plate glass. In the conventional type of procedure the eye and hand of the operator are between the image and the light source. This necessitates considerable obliquity of the line of vision relative to the surface of the paper and leads to inaccuracy of working both for geometrical reasons and from the introduction of shadows.

By the use of transmitted light it is possible throughout to maintain the eye and the point of the cutting instrument at right angles to the plane of the image, and no shadows can be introduced. Accuracy of cutting is further facilitated by the use of a rigidly supported glass screen at the eye level of the operator in a comfortable sitting position.

Acknowledgements are made to Prof. Waterston for kindly informing one of us (E. W. P.) that ready-made wax plates are now commercially available.

THE PRESENCE OF A FRONTO-PALATINE ARTICULATION IN ABORIGINAL AUSTRALIAN SKULLS

By K. F. RUSSELL
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The presence in the Australian Aboriginal skull of an articulation between the orbital process of the palatine bone and the frontal bone in the medial wall of the orbit has not, so far as I can elicit, been described.

This abnormality occurs (Fusari, 1897; Quain, 1915; Poirier & Charpy, 1931) as: (1) a prolongation upwards of the orbital process of the palate bone to articulate with the frontal, (2) a prolongation downwards of the frontal to articulate with the orbital process of the palate bone. These prolongations occur behind the orbital plate of the ethmoid, and may thus interrupt the normal spheno-ethmoidal contact.

Fig. 1. A fronto-palatine articulation of type I in its complete form (left side).

Fig. 2. Complete type I (right side).

Regarding the first type, Le Double (1906) states that, according to Grüber, this abnormality occurs in about 10 % of skulls, that it has been observed by Fusari in 22 out of 250 skulls, and by himself in 18 out of 200 skulls. Of the second type the same author quotes Grüber as stating that this abnormality is more rare. It was found in only five out of 250 skulls examined by Fusari (always on the right side), and only once (on the left) in 200 skulls examined by himself.

The presence of an abnormality of the first type in an Aboriginal skull in the possession of one of our students (Fig. 1) led me to examine the skulls in the bone room of this department.

In all 182 skulls were examined; of these 26 were Melanesian and none showed the abnormality. Eight were New Zealand Maori skulls; among them was one example of type 2 of this abnormality. 148 were Australian, among which the abnormality was distributed as follows:

Type I

Prolongation upward from the orbital process of the palate bone.
Complete, left side, 1 skull (Fig. 1).
Complete, right side, 1 skull (Fig. 2).
Partial, left side, 2 skulls.
Partial, both sides, 1 skull.

Type II

Prolongation downward from the frontal bone.
Partial, left side, 1 skull.

The skull which had the partial type I on both sides had also a complete subdivision of the left parietal bone into two elements. This skull was the subject of a paper by R. J. A. Berry in this *Journal* (1909).

Two skulls of the Australian group, though they did not show the abnormality under consideration, showed division of the orbital plate of the ethmoid bone.

Since the Department possesses only a relatively small number of skulls of white races, no comparison with the latter was attempted. However, in this small series type I was observed in 2 skulls, partial (right side) in a French skull, and partial (both sides) in a white skull of unknown provenance.

SUMMARY

1. The presence of an articulation between the orbital process of the palate and the frontal bones in the medial wall of the orbit is described in the Australian Aboriginal skull.

2. The abnormality was found in 5 out of 148 Australian skulls, and in 1 out of 8 skulls of New Zealand Maoris.

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PERICARDIO-PERITONEAL COMMUNICATION. DESCRIPTION OF A RARE TYPE OF DIAPHRAGMATIC HERNIA

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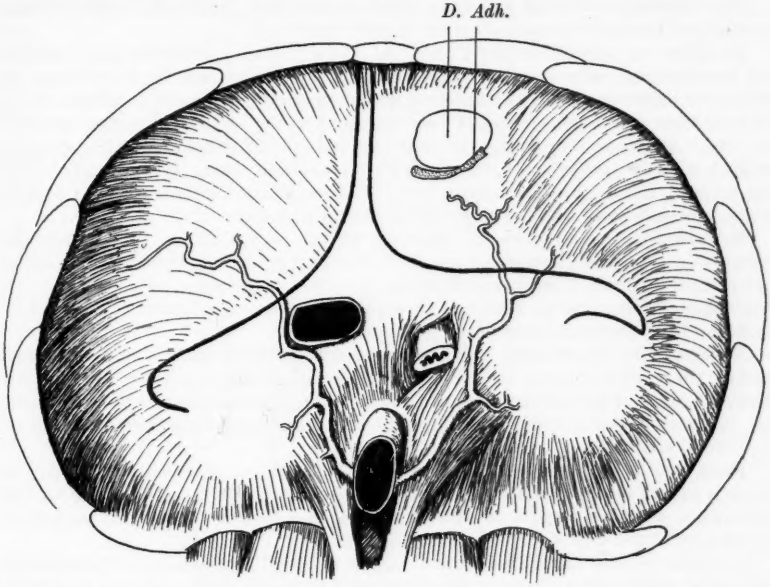
Although cases of diaphragmatic hernia are by no means uncommon, the following specimen is described because of the extreme rarity of the type of which it is an example.

In the course of routine dissection of the body of a 63-year-old Russian, recorded as having died from general paralysis, an aperture in the diaphragm was found forming a communication between the pericardial and peritoneal cavities.



Diaphragm from below. *D.* defect; *Adh.* adhesion band between liver and diaphragm. The heavy black line shows the attachment of the anterior parts of the coronary ligament and of the falciform ligament. The posterior parts are omitted for simplicity.

The opening was oval in outline, 3 cm. in transverse, and 2·3 cm. in antero-posterior diameter, lying in the anterior leaf of the centrum tendineum of the diaphragm where this is fused to the floor of the pericardium. It lay just to the left of the middle line, with its anterior border 2 cm. behind the posterior surface of the sternum. Seen from the abdominal aspect, it lay well to the left of the falciform ligament of the liver, and some way anterior to the left part of the coronary ligament. The borders were thin, smooth, and rounded anteriorly and laterally, while the posterior part of the circumference had a thin sharp border. The pericardium was directly continuous with the peritoneum all around the margins of the aperture.

Seen from the pericardial aspect, the posterior two-thirds of the aperture were occupied by a low, smooth, rounded process of the liver projecting not more than 4 mm. above the level of the surrounding floor. The anterior part of the aperture exposed some of the great omentum, which, however, had apparently not herniated through the orifice.

The heart was apparently normal externally, but there was a small patent and valvular foramen ovale such as is not uncommonly encountered in the course of routine dissections. The visceral pericardium showed a slight pearly opacity over the area of contact with the exposed liver. The aorta showed evidence of syphilitic aortitis, but otherwise there was no significant abnormality of the heart.

The pericardium, apart from the aperture in the floor, appeared normal in every respect and clothed the heart with the usual accuracy, suggesting that at no time can there have been any significant herniation of abdominal contents into it. There was no sign of a patent pleuro-pericardial canal, and the phrenic nerves ran their usual course.

The diaphragm showed no other abnormality, and the lumbo-costal triangle (foramen of Bochdalek) was closed by muscle tissue.

The liver was slightly smaller than usual, the left lobe in particular being reduced and terminating postero-laterally in a thin sheet which formed the base of the left triangular ligament. Near the anterior border of the diaphragmatic surface of the left lobe there was a low process of liver tissue extending on to the anterior border of the lobe, and accurately fitting the posterior part of the diaphragmatic deficiency, through which it projected into the pericardium for about 4 mm. Immediately behind this process, the liver was adherent to the diaphragmatic peritoneum by a narrow, tough adhesion-band running along the posterior periphery of the aperture.

Microscopical examination of the liver in the region of the process revealed evidence of syphilitic cirrhosis of the liver, thickening of the overlying peritoneum, and a narrow band of organized fibrinous exudate at the point of adhesion to the diaphragm. There was no evidence to suggest that the aperture could have been produced by a previous gummatous perforation.

No significant abnormality could be detected in the other thoracic and abdominal viscera, there were no scars on the skin to suggest a penetrating injury, and the ribs showed no sign of antecedent fracture. None of the other abdominal viscera showed any evidence to suggest that they might at some time have been herniated into the pericardial cavity.

From lack of evidence of any other possible aetiology, it appears probable that this is a case of hernia diaphragmatica spuria centralis congenita of the liver into the pericardial cavity, in itself a rare condition, but it is further remarkable on account of the very slight degree of herniation present.

A general survey of the literature available revealed that examples of pericardio-peritoneal communications have been described in five instances in man, four in the dog, and one in the ass. Of the examples in human subjects, four were of the false variety and were found in males past middle age, as in the present case, while one was a true hernia with sac in a new-born infant of unspecified sex. In all these cases there was a considerable degree of herniation of abdominal contents into the pericardial cavity. Of the examples reported in animals only one, reported by Stoeber (1912), showed evidence of the former existence of a hernial sac.

In the present instance, the lack of herniation of abdominal viscera is probably explicable on the grounds that, although the condition was probably present in some degree at birth, during most of the man's life it must have been shielded by overlying liver, which only latterly began to retreat and expose the deficiency as the cirrhotic process caused general reduction in size of the liver. This contraction during life would be further increased post-mortem by the method of injection employed in preparing the subject for the dissecting room. That contraction of the liver could effect an enlargement of the aperture is suggested by the firm adhesion of the liver to

Table I. *Pericardio-peritoneal communications*

Author	Date	Age	Sex	Type	Herniated structures	Associated abnormalities	Origin
(A) In Man:							
de Cardenal	1903	58	M	False	Great omentum. Loop of transverse colon	Omentum adherent to margins of gap. Pericardium large. Heart large and distorted. Loop of colon strangulated	Congenital
Martland	1909	70	M	False	Great omentum. Loop of transverse colon	Omentum adherent to margins of gap. Pericardium large, with thick walls	Congenital
Keith	1910	50	M	False	Process of great omentum	—	Possibly traumatic
		47	M	False	Process of liver	—	Possibly traumatic
		New-born ?		True	Process of liver	—	Congenital
O'Brien	1939	63	M	False	Process of liver	Liver adherent to margin of gap. Passable foramen ovale. Coincident lues	Congenital
(B) In dog:							
Antonio*	1901	?	?	False	Great omentum	Omentum adherent to heart	Congenital
Petit	1902	?	?	False	Coils of small gut	No quadrate lobe of liver	Congenital
Joest*	1904	Old	?	False	Great omentum	Pericardium greatly enlarged	Congenital
Stoeber	1912	?	?	? True	Process of liver	Pericardium greatly enlarged. Lungs reduced	Congenital
(C) In donkey:							
Forgeot*	1903	?	?	False	Process of liver	—	Congenital

N.B. Authors marked * are quoted by Joest (1912).

the posterior margin of the deficiency and by the firm but attenuated appearance of the left lobe and triangular ligament, which doubtless acted as a point of counter-traction.

This explanation may account for the lack of herniation in this peculiar example of a rare condition, but there is as yet no satisfactory explanation of the primary embryological aetiology of these curious defects.

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Anatomical Society of Great Britain and Ireland

PROCEEDINGS

June 1939

RECORDED BY

A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

JUNE 1939

The Summer Meeting of the Society was held in the Department of Anatomy, University of Glasgow, on Friday and Saturday, 16 and 17 June 1939, beginning at 10 a.m. each day, with the President, Professor ALEXANDER LOW, in the Chair.

Professors D. M. BLAIR and F. DAVIES read a paper on "The conducting system in the human and in the calf heart".

The topography of the sinu-atrial and atrio-ventricular nodes, and of the atrio-ventricular bundle and its limbs, has been studied in interrupted serial sections of the human and the calf heart, stained in bulk by a modified pyridine silver technique. The main mass of the sinu-atrial node covers the top of the right atrium parallel with the crista terminalis and medial to the superior caval orifice. Nodal prolongations descend in front and behind this orifice; in man the anterior prolongation is the longer, in the calf the posterior, reaching indeed the upper edge of the inferior caval orifice. Sinu-atrial nodal fibres are continuous with the musculature of sinus and auricle, but were not observed to manifest any direct continuity with the atrio-ventricular node.

This latter occupies the lower posterior part of the interatrial septum in front of the coronary sinus orifice; its fibres are continuous above with the musculature of both atria and below with the atrio-ventricular bundle. The right limb of this bundle does not branch until the moderator band is reached, whereas the left limb spreads and divides during its descent. No Purkinje fibres were detected in either the human or in the bovine atrium. The calf's atrio-ventricular bundle and its limbs are composed entirely of Purkinje fibres; in the human heart such fibres appear first in the right limb just above the moderator band and in the left limb at a higher level. Purkinje fibres in the calf heart exhibit a subendocardial distribution from below upwards in both the free and the septal ventricular walls. In the human heart, though a similar subendocardinal network occurs, no penetration of Purkinje fibres into the ventricular myocardium could be determined. Attention has been paid to the distribution of nerve cells and nerve fibres in relation to the cardiac conducting system: such distribution is rich in the calf, poor in the human heart. The calf heart revealed, at various ventricular situations, patches of muscular tissue reminiscent in structure of the atrio-ventricular node, and lying close to, though not actually continuous with, the subdivisions of the bundle limbs.

Professor DAVIES commented further upon this paper. He emphasized the dissimilarity of structure of the human and bovine sinu-atrial and atrio-ventricular nodes, the absence in either species of any direct specialized connecting tissue between sinu-atrial and atrio-ventricular node, and the difficulty of interpreting pathological changes in the right limb of the human A.-V. bundle wherein Purkinje fibres were strictly confined to the lower half only. The physiological meaning of the various histological peculiarities of the human conducting system remained still uncertain: possibly individual dif-

ferences and age changes were factors to be reckoned with in the interpretation of histological findings.

Professor WATERSTON remarked upon the ease with which the sinu-atrial node is demonstrable in the ox heart, wherein it occupies the entire thickness of the atrial wall. Subendocardial lymph spaces around the limbs of the A.-V. bundle, so readily demonstrated by injection in this species, appeared to be absent from the human heart, judging by the failure attendant upon similar injection methods. Professor BLAIR, replying, stressed the relatively less extent of the human sinu-atrial node as compared with the bovine node.

Professor F. DAVIES and Dr E. T. B. FRANCIS exhibited a cinematograph film on "The rhythm of the heart", based on physiological and experimental preparations of the salamander heart.

Professor F. DAVIES and Dr E. T. B. FRANCIS read a paper on "The conducting (connecting) system of the heart of the salamander (*S. salamandra*)". The heart of the salamander was chosen as a basis for a comparative study of the connecting systems of the hearts of poikilothermal vertebrates, partly because a previous study of the anatomy of the salamander as a whole (E.T.B.F.) revealed its generalized nature, and also because of the large size and complete independence of the sinus venosus. The principal morphological peculiarities of the heart are the position of the atria (entirely to the left of the bulbus), the close connexion between the pulmonary vein and the sinus venosus (the vein actually passing through the dorsal wall of the sinus on its way to the left atrium), and the entry of the pulmonary vein into the left atrium through the atrial septum and not through the atrial wall.

The heart muscle is striated throughout and consists of very large fibres with large sausage-shaped nuclei. There is no essential difference between the structure of the fibres in the several chambers of the heart, or in the junctional zones (s.-a. and a.-v. junctions). There is, however, a difference in the direction of the fibres at these sites; for whereas the walls of the several chambers of the heart consist of an irregular meshwork of fibres, the junctional zones consist of perfectly regular circular fibres arranged sphincterwise around the openings. At the bulbo-ventricular junction also, the ventricular longitudinal fibres become continuous with those of the bulbus wall, which, however, consist of regular circular muscle throughout.

The size of the fibres throughout the heart is strikingly uniform. The largest fibres are found at those places where the regular circular arrangement is followed, viz. s.-a. and a.-v. junctions and bulbus wall. The average sizes of the fibres in the walls of the sinus, atria and ventricle are practically identical. There is nowhere in the heart of the salamander any specialized junctional muscle such as described by Keith and Mackenzie in fishes, other amphibia, and reptiles. Whilst detailed studies of sections of the hearts of the eel, gurnard, tortoise, Mississippi alligator and *Sphenodon* still await completion, preliminary inspection of such sections has failed to reveal in these forms the presence of any specialized muscle at the sinu-atrial and atrio-ventricular junctions. Confirmation of the absence of specialized muscle in the lower vertebrate heart will entail the interpretation of the conducting cardiac muscle of birds and mammals as a neomorphic structural development in response to the physiological requirements of the more rapidly beating heart of the homoiothermal vertebrate.

Professor BLAIR commented upon the concomitant absence of specialized conducting tissue and of a coronary circulatory system in the salamander heart. Dr ADAMS said he had failed to find any specialized muscle tissue in the heart of *Sphenodon*, at the junctional regions or elsewhere. Dr PARSONS discussed the extension of ventricular muscle into the bulbus cordis in connexion with the physiology of bulbus contraction. Dr TUDOR JONES enquired concerning the age of the material used and the methods of its preparation.

Professor DAVIES replied, detailing the histology of the walls of both truncus and bulbus cordis and correlating this, as far as is possible, with the functional activity of the heart.

Drs P. BACSICH and G. M. WYBURN read a paper on "Cyclic variations in the vascular architecture of the guinea-pig uterus" which will be submitted for publication in the *Journal*.

Professor NICOL said the present findings (of antimesometric increased vascularity in the cavy uterus) confirmed his own similar observations made in this species some years previously. Dr GRAY asked whether true hyperaemia could be satisfactorily distinguished from a new growth of subendometrial blood vessels. Dr BACSICH, in reply, regarded the subepithelial vascular plexus as a constant and characteristic anatomical feature of the guinea-pig uterus. No vascular injection had been made of the uteri used, so that existent vessels were dilated solely by natural agency.

Dr W. E. ADAMS read a paper on "The branchial derivatives of *Lacerta*" which will be submitted for publication in the *Journal*.

Professor E. J. R. EVATT read a paper on "The bregma as a central point for cranial measurements" which will be submitted for publication in the *Journal*.

Dr A. J. E. CAVE read a paper on "A new reconstruction of *Australopithecus*". A generalized reconstruction of the Taungs fossil anthropoid (*Australopithecus africanus*) was made as follows. The missing parts of cranial vault and mandible being first restored on a cast of the original fossil, the probable depths of the subjacent soft tissues (estimated from measurements of immature chimpanzee material) were indicated at various points thereon by pieces of plasticine of appropriate height; the general contour of the head was then built up in the same material to the surface level indicated by these guides. The external nose, eyes and pinnae were modelled on the basis of the chimpanzee type of structure. The cast exhibited, and made from the plasticine model, demonstrates the salient features of Australopithecine anatomy. It depicts a paedomorphic type of anthropoid, closely related to the chimpanzee, but manifesting a greater relative development of the frontal and parieto-occipital regions of the brain and still retaining the facial and cranial features of a young animal. The cast differs considerably from the first reconstruction by Dart, but is not dissimilar from the restorations published by Keith and Elliot Smith.

Professors C. M. WEST and J. H. DIBLE read a paper on "A very early human embryo" which will be submitted for publication in the *Journal*.

Professor HAMILTON asked what evidence the specimen afforded as to the

formation of early mesoderm, and also whether the embryonic entoderm was of uniform structure throughout. Dr INKSTER enquired as to the criteria employed in distinguishing amnion from yolk sac. Dr BACSICH considered hyperaemia of the uterine mucosa to precede the embedding of the ovum. Professor WEST in reply stated that the primitive mesoderm was possibly delaminated from the inner wall of the trophoblast; the entoderm appeared to be fairly uniform, but still required particular study. The distinction between amnion and yolk sac presented difficulties: the latter was usually (though not invariably) the larger of the two cavities, and had so been interpreted in the present instance. It was impossible to decide in this specimen whether uterine hyperaemia occurred before or after implantation of the ovum: such vascular engorgement was not known to be particularly localized in the human uterus.

Professor C. P. MARTIN and Dr H. D. O'BRIEN exhibited a cinematograph film illustrating "Movements at the shoulder-joint".

The film demonstrated the normal active range of movement at the shoulder-joint in the living subject, which was correlated with passive movements obtained in the dissected shoulder of a fresh, uninjected cadaver. The pictures demonstrate the lateral rotation of the humerus in full elevation of the arm. This rotation is independent of muscular activity and is due to the coracoacromial arch: this structure prevents humeral abduction beyond a certain point by impeding movement of the greater tuberosity of the humerus. The tuberosity is able to slide under the arch only by travelling backwards and thus imposing lateral rotation upon the humerus.

Professor LOCKHART discussed the film at length and exhibited a second film, illustrating movements of the shoulder-joint and pectoral girdle in young women, in support of his remarks. Professor APPLETON described scapular movements executed under a new muscular co-ordination mechanism, in cases of paralysis of the trapezius muscle. Dr WHILLIS regarded movements obtained in the cadaver as relatively uninformative owing to the absence of tone. In most subjects the medial epicondyle pointed forwards in full elevation of the arm, for which movement the musculature, not the ligamentous structures, was responsible. The "plane of the scapula" still remained a useful and practical conception.

Dr O'BRIEN replied that the cadaveric experiments resulted in the same final effect on the arm as that obtained in the living body, that the supraspinatus muscle depresses the greater tuberosity during the ultimate stages of arm elevation (as witnessed by the final sinking of the humeral upper end) and that the common clinical occurrence of atrophy of the supraspinatus tendon corroborates the mechanism of arm movement indicated by the cadaveric experiments.

Dr M. A. MACCONAILL read a paper on "Structure and function in joint-series (Gelenkketten)" which will be submitted for publication in the *Journal*.

Professor APPLETON read a paper on "The measurement of anatomical changes in the thorax in the course of physical education", and another paper on "Pelvic obliquity in the living subject", which will be submitted for publication in the *Journal*.

Reply was made to Professor DURWARD that all the subjects examined during investigation of thoracic changes were females of an average age of

19 years. To some slight degree the different types of bodily habitus were reflected in the results obtained, but the matter was still open to further enquiry.

Professor J. C. Brash read a paper on "Vital staining of bone with hydroxyanthraquinone derivatives".

It has long been known that madder owes its property of staining bone to the anthraquinone derivatives alizarin (1 : 2 dihydroxy) and purpurin (1 : 2 : 4 trihydroxy); but, contrary to the usual statement, the purpurin content of madder is more important from this point of view than the alizarin.

A number of other hydroxyanthraquinone derivatives have been fed to rats in the search for a bone-staining substance with distinctive colour to facilitate the study of bone-growth, and in order to determine the relation, if any, between chemical structure and the property of vital staining of bone.

The ten derivatives used range from 2 : hydroxy *A*/*Q* to 1 : 2 : 4 : 5 : 7 : 8 hexahydroxy *A*/*Q*. In addition to alizarin and purpurin three others have given positive results, each producing a characteristic colour.

Vital staining of bone appears to be related in a general way to chemical structure; the effective derivatives have hydroxyl in the 1 : 2 positions, and there is a graduation of colour from pink through purple to blue with the addition of hydroxyl in the 4, 5 and 8 positions.

A series of sixteen more complex dyes have also been tested. Some of them have proved toxic, and only one of them—an alizarin sulphonic derivative with hydroxyl in the 1 : 2 positions—has given a positive result with a colour similar to but distinct from that produced by alizarin itself.

Dr R. Walmsley read a paper on "The development of the patella" which will be submitted for publication in the *Journal*.

Dr Whillis asked for details of the state of the joint-disc at the stage immediately following its minimal reduction. Professor Blair quoted corroborative results from the experimental work of Dr Roberts, who has found the patella to develop within the quadriceps tendon, to be recognizable in embryos of 20 mm. or so, and to develop relatively high up in relation to the femur. Dr Walmsley, in reply, described the gradual ontogenetic descent of the patella and its consequent successive relationships to the front and lower surface of the femur.

Dr W. Walls read a paper on "The central nervous system in a case of congenital absence of one leg".

Examination was made of the spinal cord, brain stem and cerebral cortex from a 75-year-old woman in whom the left lower limb below the knee was congenitally absent. Asymmetry was found in the spinal cord as follows: the white matter of the posterior columns was quantitatively less on the left side at lower levels, and at higher levels it was apparent that the gracile tract had received less than the normal complement of fibres. The white matter of the anterior and lateral columns presented no obvious quantitative difference between the two sides. In the sacral region the grey matter of the left side was quantitatively less than on the opposite side and certain cell groups appeared to be absent. The brain stem appeared quite symmetrical, and in the cortex, Betz cell counts from both precentral gyri revealed no observable difference between the two hemispheres.

Dr L. M. Dickson read a paper on "The development of nerve endings in the respiratory muscles of the sheep", which will be submitted for publication in the *Journal.*

In the subsequent discussion Professor Brash asked whether any correlation had been noted between the development of nerve-endings and the onset of muscular activity; Professor Davies enquired whether such endings became functional before becoming argentophile; Professor Blair remarked upon the histological nature of end-plates, and Dr Tudor Jones sought information upon the innervation of intrafusal fibres. Mr Harpman referred to the difficulty of staining muscle-spindles by the methylene blue technique and to the occurrence of muscular movements in rat embryos at a stage prior to that at which nerve-endings are stainable. Dr Dickson replied.

Dr G. A. G. Mitchell read a paper on "The nerve supply of the stomach", which will be submitted for publication in the *Journal.*

Professor Davies urged the histological study of the numerous vagal branches and connexions described: the details of gastric innervation submitted in this communication fully explained the failure of such operative procedures as denervation of the left gastric artery in the attempted cure of achalasia. Dr Walls instanced examples of the vagus assuming the form of a single nerve below the lung root in the posterior mediastinum. Dr Mitchell, in reply, intimated his intention to carry out histological examination of the vagus and its several connexions: twice only in 36 subjects examined had he observed the single-strand type of infra-pulmonary vagus.

Dr S. Sunderland read a paper on "The distribution of commissural fibres in the corpus callosum of the macaque monkey", which will be submitted for publication in the *Journal.* The author answered Mr Harpman that no experimental proof was available concerning the presence of commissural fibres from the insula, and Dr Cave that the localization of such fibres had not yet been determined in the monotreme and the marsupial brain. Professor Lockhart referred to his personal investigations of the course of anterior commissural fibres in the hedgehog, cat, rabbit and macaque brains and to the difficulties of localization encountered.

Dr S. Sunderland read a paper on "The projection of the cerebral cortex on the pons and cerebellum", which will be submitted for publication in the *Journal.*

Dr B. Schlesinger (introduced by Professor Le Gros Clark) read a paper on "The venous drainage of the brain with special reference to the Galenic system".

In a series of monkeys one of the small veins of Galen was blocked experimentally in order to trace the distribution of its tributaries by means of the temporary hyperaemia, and in order to study their anastomotic connexions. Apart from anastomoses formed by capillary loops and precapillary anastomoses in the white matter, a collateral circulation is established by relatively large veins which directly connect the tributaries of the veins of Galen with the cortical veins on the surface of the brain. These venous channels, which in general follow the stream of callosal fibres, were also demonstrated in the human brain by injection methods.

Collateral circulations in the basal ganglia are formed only by the very dense capillary network which connects the superior thalamic and superior lenticular with the inferior thalamic and inferior lenticular vessels. In relation to the venules of the caudate nucleus, numerous small haemorrhages occur as a result of blockage of the corresponding vein of Galen.

Collateral drainage in the chorioid plexus is readily allowed by the rich plexiform arrangements of its veins, and no hydrocephalus occurred in the experiments.

Concerning the effects of compression of the vena magna Galeni upon the rate of flow of the cerebro-spinal fluid, the author stated he had no observations to put forward; different workers upon this topic had drawn different, often contradictory, conclusions. Clinically, hydrocephalus results not from Galenic vein compression, but from compression of the Sylvian aqueduct.

On behalf of Professor E. LANDAU a cinematograph film ("Du cerveau animal au cerveau humain") was exhibited at the conclusion of the meeting.

DEMONSTRATIONS

At this Meeting of the Society the following Demonstrations were given:

(1) E. J. R. EVATT: (*a*) Abnormal pars basilaris of occipital bone. (*b*) Abnormal acromion.

(2) J. C. BRASH: Specimens illustrating vital staining of bone with hydroxyanthraquinone derivatives.

(3) J. BRUCE and R. WALMSLEY: Specimens illustrating effects of experimental excision of patella in rabbits.

(4) E. P. REAGAN: (*a*) Thick sections of human embryos injected and cleared by special method (see *Univ. Calif. Publ. Zool.* **28**, no. 18, 1926).

(*b*) Stereoscopic views of embryos similarly treated (prepared by Professor J. A. Long, Univ. Calif.).

(5) A. J. E. CAVE: Restoration of *Australopithecus africanus*.

(6) T. H. BRYCE: Bryce Collection of human embryos.

REVIEWS

A Synopsis of Regional Anatomy. By T. B. Johnston, M.D., Ch.B. Fourth edition. (London: J. and A. Churchill Ltd.) 1939. Pp. xxi+462, 17 figs. Price 12*s.* 6*d.*

It is a tribute to the undoubted popularity of this book that another edition is now called for. While there is no major alteration in the text and while the author still quite rightly refuses to include any illustrations in the greater part of the work, he has added some very clear and helpful diagrams to those which appeared in previous editions in the section that deals with the central nervous system. As he insists very properly, this book should not be consulted at all until the actual work of dissection has been completed and revision is being undertaken. If the student will bear this in mind he will find much to help him in this volume.

The Tissues of the Body. By W. E. Le Gros Clark. (Oxford: Clarendon Press.) 1939. Pp. xi+372. Price 15*s.*

The usual thought which books designed for students of anatomy in this country bring to mind is an endless succession of works on topography, occasionally interrupted by one on embryology. It is a comfortable thought, one which restricts the scope of the subject almost to a defined number of things that can be dealt with by means of scalpel and forceps. That it is also an uninspiring thought goes without saying, and its dreariness is made acutely plain when something different appears within the anatomical orbit. Prof. Le Gros Clark's book is different and it has just that effect. For here is a book which completely denies the limitations of anatomy that have been imposed upon it by the greater part of its practice in Great Britain during the past thirty years. It boldly redefines the scope of the subject as being not only the study of the structural organization of the body, but also the investigation of the "conditions under which this organization is achieved during embryonic and post-natal development" and of "the processes whereby it is maintained after the body has reached maturity". If this book fails to have a considerable influence on the reorientation and future development of anatomy in this country, it is difficult to see what will succeed.

The essential purpose of the book is to discuss the meaning of the terms which form the units of anatomical discussion. It is divided into twelve sections which deal respectively with the cellular basis of tissues, their development in the embryo, connective tissue, bone, muscle, the tissues of joints, blood-vessels, blood, lymphatic tissues, mucous membranes and glands, skin, and the tissues of the nervous system. Each of these sections is not only an introductory account of the subject with which it deals, but also includes a review of recent studies, experimental and otherwise, bearing on the problems under discussion. Each section is subdivided according to the needs of its particular subject; for example, the section on bone is organized under the following heads: "the structure of bone; the periosteum; ossification; the role of the osteoblast and osteoclast; the growth of bone; epiphyses; times of ossification; anatomical methods of studying bone growth; the mechanics of bone; the self-differentiation of bone; the influence of mechanical factors on the shape of a bone; factors initiating the process of ossification; some metabolic factors in bone growth; and the vascular and nerve supply of bone." That on skin, again, is treated under the subsections "the structure of skin; flexure lines; pigmentation of skin; hair; cutaneous glands; nails; the innervation of skin; the blood supply and lymphatic

drainage of skin; and the regeneration of skin." References are given to the more prominent papers on which the chapters have been based, and the whole is admirably illustrated both with half-tone reproductions of photographs and photomicrographs, and with schematic diagrams whose simplicity and effectiveness leave nothing to be desired. They are an excellent accompaniment to a text whose clarity of exposition presents the subject of anatomy not as just a storehouse of topographical facts, but as an exciting field of research into questions which are vital to the understanding of almost every growing aspect of medical science.

The detail of a book of this kind requires no review. Its effect and intent are however plain. Let a student beginning anatomy turn to any page, and he will find information of absorbing interest which is not only missing in his usual text-books, but without which most of the facts of those text-books can have little significance except as tests of memory. The information which Prof. Le Gros Clark has brought together and the issues which are the concern of topographical works are clearly complimentary, and there is not the slightest doubt that the wearisome aspects of dissecting-room teaching would be much simplified and invigorated were they combined with instruction in those phases of the subject dealt with in *The Tissues of the Body*. How, one may well ask, can a student be expected to have the interest and capacity to remember the lymphatic drainage of the leg or mediastinum when the very term "lymphatic drainage" may mean practically nothing to him, when he has no knowledge of the structure of lymphatic vessels and plexuses, of their mechanism of absorption and their relation to tissue spaces, of the methods by which they are studied, of their relation to lymph nodes, and of the structure and function of lymph nodes? How indeed, and yet this is what most of us had to do ourselves, and what most of us still expect our students to do. Prof. Le Gros Clark's book shows a better way.

There may be some, particularly among those who are not professional anatomists, who will feel that the book includes topics that are not strictly within the province of anatomy, and that such topics were considered because of some peculiar and individual appreciation by the author of what the subject comprises. Nothing could be further removed from the truth. Anatomy was never just concerned with the description of structural relations. It was always something more. Even when regarded as a discipline relating purely to morphological description, it was rich with the vigour and inspiration of evolutionary theory. To limit anatomical teaching to structural relations is to turn the subject into simple vocational instruction. And if anatomy were to limit itself to that, it would cease to fulfil more than its most elementary function, for medical science every day reaches beyond the confines of the surgical practice to which such anatomical teaching relates. Every advance of medicine makes new demands of anatomy which far transcend the straight-forward teaching of the dissecting room. In a sense, therefore, most of the topics discussed by Prof. Le Gros Clark are also anatomy's more recent contributions to medical science. Furthermore, the methods by which these contributions have been made, the methods of experimental biology and microscopic anatomy, are as much the methods of anatomy as is simple dissection. The curious fact that microscopic anatomy is usually regarded in Great Britain as a part of physiology and not of anatomy is, as is now well recognized, the result of purely fortuitous historical circumstances, and in no way does it obscure the real connexion of histology and cytology with the science of anatomy. Similarly the fact that physiology always makes use of the experimental method does not imply the *non sequitur*, which one still sometimes hears argued, that this method has no place in anatomy. In reality the experimental method is by far the most important tool of anatomy to-day, a fact which is admirably demonstrated by Prof. Le Gros Clark's book. To hide it from the student is sheer irresponsibility. Progressive schools of anatomy long ago abandoned the barren fields of exploration into which purely morphological conceptions of the

subject led. The subject grows to-day mainly out of the experimental investigation of the conditions under which structural organization is achieved and maintained. If its growth in this country is to be stimulated, if anatomy is to take advantage of the great achievements which lie dormant within it, then anatomical teaching, while it continues to deal with the structure of the body, must also be re-orientated in the directions along which medical science is progressing. The way is clear, and Prof. Le Gros Clark's book gives a brilliant lead.

Synopsis of Surgical Anatomy. By A. L. McGregor, M.Ch., F.R.C.S. Fourth edition. (Bristol: John Wright and Sons, Ltd.) 1939. Pp. xvii + 664, 648 figs. Price 17*s.* 6*d.* net.

We welcome another edition of this useful book. While there is little change from previous issues, it admirably maintains its deserved reputation as a mine of accurate information, much of which can only be gleaned with difficulty from the standard surgical or anatomical text-books. The clear diagrammatic figures add much to what is perhaps one of the best manuals of applied anatomy which are at present available.

A Manual of Practical Anatomy. Part II. The Thorax and Abdomen. By Thomas Walmsley. 2nd ed. (London: Longmans, Green and Co.) 1939. Pp. 319, 132 figs., Pl. VII. Price 12*s.* 6*d.* net.

The first edition of this book appeared in 1921. In this, the second volume of the second edition, new matter has been added, especially with regard to those parts which are of practical importance to the future doctor, and seven good X-ray plates have been inserted. Might we suggest, however, that more careful proof reading would avoid minor but irritating errors such as occur in the list of Plates and again in Fig. 16? The diagrams, too, could be drawn with a greater concern for accuracy without losing any of their admirable simplicity. As examples, one might cite Fig. 86, where it is impossible to tell where the diaphragmatic area ends, and the lateral arcuate ligament has not been marked with any lettering. Again, in Fig. 99, the trachea is represented as being in contact with the left lung, and the subclavian artery is shown on an anterior plane. Fig. 105 has the sanctity of age, but it is a source of endless muddle to students, who naturally infer from it that the transverse sinus of the pericardium lies above the heart, with the atria *below* and the great arteries *above* it. Might one also plead that the highly overrated pelvic fascia should no longer be described as a structure independent of muscles and viscera as in Fig. 20 and p. 42?

Handbuch d. vergl. Anatomie d. Wirbeltiere. Band 5 geheftet RM. 132. H'ldr. RM. 138. Urban & Schwarzenberg, Berlin and Vienna.

This volume of the Handbuch fully maintains the high standard of those previously published. The first part is devoted to the skeleton, muscles and nerves of the extremities. There are two sections on the musculature of the head, one concerning the somatic, and the other the visceral muscles. As these are widely separated in the volume, it makes cross reference work difficult. Articles on the electric organ, the accessory glands of the urogenital system, and the structures surrounding the mouth cavity, are well arranged and complete. The section most outstanding for arrangement of subject material and method of treatment is that on the urogenital system by van den Broek, van Vordt and Hirsch, all of Utrecht. References are found at the end of each article and are well selected.

THE NATURE OF THE SOFT PALATE

By FREDERIC WOOD JONES

INTRODUCTION AND NOMENCLATURE

In attempting to determine the true nature of any anatomical structure two factors, which at first sight may seem trivial, must be taken into account. In the first place our conception of any anatomical entity is likely to be influenced by the form of the entity that was first described and to which the distinctive name of the structure was first given. This, in its turn, will obviously depend upon what was the type of animal in which the structure was originally described. There are, as is well known, many instances of classical errors in human anatomy caused by the fact that descriptions of certain organs were founded originally upon the dissection of specialized mammals representative of Orders widely separated from the Primates. Some of these errors are, unfortunately, not cleared completely away from human descriptive anatomy even at the present day. On the other hand, the fact that so much anatomical description was first stereotyped from human dissection has led to a loss of the true conception of the nature of certain structures that have their complete expression only in other mammals.

The second factor that must be taken into account is the circumstance that the nomenclature will almost certainly be standardized in accordance with the type of structure to which it was first applied and that, therefore, it may be completely misleading when applied to the structure as seen in more perfected condition in other animals. The preservation of a nomenclature that is not applicable to the true nature of a structure is bound to lead to a lack of realization of the essential characters of any part or organ of the body. Since an incorrect nomenclature may so easily lead to a complete misconception of the nature of a structure, it is obvious that the perpetuation, or the alteration, of a terminology is a matter of considerable importance when the question of scientific accuracy is considered. I have elsewhere in this *Journal* (1939) called attention to the steady deterioration in the precision and accuracy of the nomenclature of the superior alveolar nerves that has taken place during the last century and, in that instance, have cited the latest (*Anat. Soc.* 1933) revision of anatomical terminology as being a retrograde step in the progress of precise nomenclature.

It is, therefore, worth while to inquire if in any way nomenclature has influenced our conception of the nature of the palate and if such changes as have been made constitute an advance in our realization of its true character or have helped to obscure it. The first step in this inquiry involves the term *pharynx*. When Galen introduced this term into descriptive anatomy he doubtless did so, as in so many other instances, from his knowledge of the anatomy of

mammals other than man. By him the term "pharynx" is used for the throat —the passage by which the food passes from the mouth to the stomach. As signifying the food passage of the throat the term "pharynx" is employed by all the older anatomists. Only a few instances of this usage need be cited. Thomas Gibson (1688) writes of "the throat or the beginning of the oesophagus called pharynx". James Keill (1703) defines "the oesophagus or gullet" as "a long, large, and round canal, which descends from the mouth" and further states that "the upper end of the gullet is call'd Pharynx". Dionis (1716) speaks of "the Pharynx, which is only the orifice of the oesophagus dilated to a great extent. 'Tis like a funnel, and some call it the gula or gullet. 'Tis seated at the bottom of the mouth that it may receive what is swallowed." Heister (1752) says "the oesophagus or gula... is a membranous canal reaching from the fauces to the stomach... its upper part is called by anatomists the pharynx". There can be no doubt whatever as to what was originally meant, and what the older anatomists understood, by the term "pharynx". There can also be no doubt that, were our knowledge of these parts still to be confined to the primitive (and more perfectly developed) condition seen in most mammals, the term "pharynx" would still be employed in this simple and unambiguous fashion. For the older anatomists there was a mouth or oral or buccal cavity leading into a funnel-shaped pharynx which passed directly into the oesophagus. Above the mouth and pharynx there was a palate which separated them from the nasal chambers above. This palate does not at all times cut off communication between the nasal chambers and the pharynx, since it has an aperture in it that leads from one cavity to the other. This older conception is the only one that can with any propriety be applied to the condition found in any primitive mammal: it is the only conception that can rightly be applied to the condition present in man. Unfortunately this correct and simple terminology has been confused by human anatomists, who, having failed to realize the true nature of the, somewhat modified, human palate, have extended the term "pharynx" to the posterior part of the nasal chambers, a region to which the term can in no proper sense be applied. How far modern usage has led anatomists astray from the true conception of the connotation of the terms may be gathered from the following statement taken from the monograph of Negus (1929): "In most mammals the oral cavity has its axis placed directly in line with that of the pharynx, if the latter can be said to exist at all." It is perhaps too late to make a plea that the terms "nasopharynx" and nasal parts or nasal portions of the pharynx should be abandoned: but it must be pointed out that their employment involves endless confusion and gives currency to a conception of the parts that is wholly erroneous. No part of the nasal chambers can possibly constitute any part of the true pharynx, for always, in all animals, they are separated from it by the palate which forms the roof of the mouth and the roof of the true pharynx. Although such a state of affairs is made abundantly clear from the merest inspection of the condition present in any primitive mammal, the typical disposition of the corresponding parts in man has undergone some

change and the true nature and entire extent of the palate have not generally been recognized by human anatomists. There is no question involved in the anatomical limits of the hard palate. As the roof of the oral cavity and the floor of the nasal cavities, it is immediately recognized and homologized in all vertebrates. It is when a definition of the limits of the soft palate is sought for that there is uncertainty in human anatomy. The soft palate, now orthodoxly known as *palatum molle*, has been variously designated velum palati, velum pendulum palati, septum palati, valvula palati, or claustrum of the palate; and by most human anatomists has been considered as that part of the roof of the mouth and "oral pharynx" that stretches from the hinder edge of the hard palate to a posterior free margin, culminating in the middle line in the uvula. From the hinder end of this palatum molle three separate folds pass to the sides of the tongue, to the pharyngeal wall and to the thyroid cartilage respectively, and these folds appear to be regarded as being much upon the same morphological level, as ridges of mucous membrane raised up by muscles passing from the palate to other parts. Few human anatomists have differentiated in marked degree the morphological significance of the so-called anterior pillars and posterior pillars, which mark respectively the palatoglossus and palatothyroideus muscles, and the third fold, which marks the true palatopharyngeus. A few exceptions may be quoted to demonstrate that some few human anatomists have recognized that the two sets of pillars are themselves on very different morphological planes and *that the posterior pillars are in reality a part of the velum palati* which in man has undergone a very definite change from the primitive mammalian condition. Winslow (1733) describes the velum palati as continuous with the posterior arch which has "a continuation with the sides of the pharynx". Monro (1831) describes the posterior pillars as "the lateral edges of the palate". Trew (1840) says "The posterior pillars constitute in fact the free border of the velum." The best description is probably that of Dionis (1716) for he says that "upon the sides of the uvula we meet with two arches,[1] which are the mouth of the Rima Nasalis: these arches consist of semi-circular fibres...when the semi-circular arches stretch themselves lengthwise, they become strait, in order to confine the air within the mouth when we blow or heave up the cheeks.". It would be difficult to furnish a better description of the parts: and the introduction of the term *Rima Nasalis* is a noteworthy detail. All these authors have realized that the posterior pillars are on an altogether different plane from the anterior pillars and that they are in fact a part of the backward continuations of the velum palati to join the posterior wall of the pharynx. Winslow even went a stage further in describing the muscles of the posterior arches (m. pharyngostaphylini = m. palatopharyngeus as distinct from the m. palatothyroideus) for he states that they "seem to form an entire arch by the union of their fibres". The description of the entire arch, by Winslow, makes the remarkable illustration of Samuel Collins (1685, Table II, Fig. 1) more easy to understand (see Fig. 1). Turning again to modern descrip-

[1] He is speaking of bilateral arches, not of the two sets of "pillars of the fauces".

tive anatomy and terminology, it would seem that in human anatomy the fact that the velum palati with the posterior pillars and the meeting of these pillars on the posterior pharyngeal wall (as will be shown later) constitute a single continuous entity has been somewhat lost sight of. Also the fact that this continuous entity forms the cephalic limit of the true pharynx has not been

Fig. 1. (Fig. 1, Table II, Samuel Collins, Vol. 2, 1685.) "*m.m.* the cavity, seated beyond the uvula, is encircled every way with conglomerated glands, which discharge themselves into it as into a common duct or cistern: this cavity receiveth air from the two cavities of the nostrils, and transmitteth it through the cavity of the mouth to the larynx, aspera arteria, and bronchia of the lungs. *n.n.* the entrance of the cavity is encircled by divers minute glands."

appreciated by human anatomists for almost two centuries. The term "Rima Nasalis" employed by Dionis for the communication between the true pharynx and the nasal cavity, made *via* the velum, is an excellent one and surely more accurate and more informative than the orthodox *pharyngeal isthmus* which is, morphologically, meaningless. If the term oropharyngeal isthmus, to denote the presumed meeting of the oral and pharyngeal cavities at the anterior pillars, is to

replace the older isthmus faucium because of the "lack of agreement concerning its precise significance" (Nomenclature report, 1933), it is surely time that some such term as nasopharyngeal isthmus, or, better still, hiatus nasopharyngeus, should be employed, instead of the wholly incorrect pharyngeal isthmus, for the aperture in the soft palate. Since the examination of the parts in any generalized mammal makes it clear that the velum always separates the nasal chambers above from the pharynx below, it is remarkable that orthodox human descriptive anatomy should still include a definition of the soft palate so strikingly incorrect as that which is current. No morphological justification whatever exists for the statement that "the soft palate is a movable fold, suspended from the posterior border of the hard palate, and forming an incomplete *septum between the mouth and pharynx*" (Gray, 1938). To define it thus is to lead to a total misconception of its morphological nature and of its function. In no mammal does the soft palate intervene between the mouth and the pharynx.

THE INTRANARIAL EPIGLOTTIS

It is impossible to discuss the true nature of the palate without some reference to the subject of the intranarial epiglottis around which a considerable amount of interest centred just over half a century ago. In vol. 23 of this *Journal* (1888–9) G. B. Howes, R. L. Bowles, Bland Sutton, Symington and others made a series of contributions to the discussion. The question had an added interest at that time in consequence of the very varying statements that had been made with regard to the intranarial position of the epiglottis in the human embryo and foetus. It was expected that a true evolutionary trend would be displayed in the human foetal condition, and only the controversial atmosphere that became woven about the question succeeded in masking this real evolutionary nature in the conditions recorded. Bland Sutton was quoted by Howes as the authority for the statement that "he had detected the truly intranarial condition in the human foetus at five months and at birth": but at the meeting of the Society held on 30 May 1889, Bland Sutton retreated in some measure from this position and stated that "he would not be responsible for the statement that the epiglottis was always intranarial in the human foetus, although it often was in the fifth month of intrauterine life". Meanwhile Symington had shown a developmental series of human embryos in which the epiglottis was in all states intrapharyngeal. The work of Bowles was adversely criticized by Dr Benjamin Howard and, as the evolutionary significance of the human embryonic condition appeared to be somewhat uncertain and the evidence cited from examination of the foetal pig unreliable, the subject became less attractive. Howes had, however, studied the problem at first hand in many mammals, but his attention was directed far more to considerations of the relative levels of the epiglottis and the velum than to the characters of the velum itself. In the whole of the discussions at this time, and in subsequent writings on this question, there has been a tendency to regard the intranarial

epiglottis merely as an epiglottis thrust up behind the posterior border of the velum palati into the nasal chambers. Such an outlook is obviously dictated by the conception of the velum as a flap, added to the hind end of the hard palate and having itself a free posterior margin, which was assumed to be its morphological termination. Howes gives no detailed description of the palates of any of the many animals in which he determined the presence of an intranarial epiglottis: moreover, after writing his first paper, he became aware of the work of Rückert, published six years earlier, and learned that his own discovery of the intranarial epiglottis in the rabbit—the discovery that started his investigations—was not original. Interest in the whole subject waned and even in the great monograph of Negus published in 1929 far more attention is paid, in this connexion, to the features of the larynx than to the condition of the velum that is associated with the intranarial epiglottis.

THE COMPARATIVE ANATOMY OF THE SOFT PALATE

It has been mentioned that one factor that may tend to obscure a realization of the essential characters of any anatomical structure is the failure to recognize the structure as it presents itself under rather different guises in varying animal types. The statement that "the soft palate, or velum palati, which is only found in mammals, is a new structure" is taken from Gegenbauer (1878), but it may be accepted as representative of the views of comparative anatomists generally. The accepted teaching is that there is originally a *primitive palate* and to this primitive palate there is added a velum palati, as a neomorph, in the mammals alone among the vertebrates. This orthodox view is well stated by Keith (1913) as follows: "In amphibians, reptiles and birds the naso-buccal channel becomes dilated to form a true respiratory nasal passage and the parts bounding the passage unite on the roof of the mouth to form the *primitive palate*." After enumerating the elements involved in the formation of the primitive palate, he proceeds: "In mammals a fourth element is added to the primitive or reptilian palate, and in this way the mouth is separated from the nasal respiratory passage and can serve the purpose of mastication and suction." The new (fourth) element added in the mammals is the velum palati and it may be mentioned incidentally that the cavity of the mouth, ever since its most primitive inception, has served the purpose of mastication and that this is certainly no new function begot by the development of a velum.

As a matter of fact, the orthodox statement that the velum palati is a new structure found only in the mammals stands in need of revision. It seems strange that such a statement should ever have been made and still more remarkable that it should still be maintained. For the velum palati was certainly recognized as being present in submammalian vertebrates as early as the seventeenth century and its presence in these classes was accepted as axiomatic by John Hunter a century later. It would seem that more recent anatomists are not content with claiming that the velum palati is a structure

peculiar to mammals, for in a recently published work on human anatomy we have the remarkable assertion that "a palate is peculiar to mammals including man" (Grant, 1937), and the context leaves no doubt in the reader's mind that the whole of the palate is included in the dictum.

THE VELUM PALATI IN REPTILES

In the Reptilia, as a phylum, the nasal chambers are separated from the oral cavity by a bony palate of the type known as the primitive palate. This primitive palate may be defined, on orthodox lines, as consisting of: (1) "a premaxillary and vomerine part developed between the nasal passages; (2) a right and left maxillary part, laid down on the lateral or outer aspect of each passage" (Keith, 1913). In the majority of reptiles the primitive palate does not extend far back into the oral cavity and the posterior orifice of the nasal chambers is therefore situated in the fore part of the mouth. In the crocodiles, however, this bony palate is of great extent and is carried backwards on the roof of the mouth by the mid-line meeting of the palatines and pterygoids. This extensive bony union on the floor of the nasal chambers causes the hiatus nasopharyngeus to be displaced backwards until it is carried to the posterior end of the cranium and is situated only a short distance anterior to the occipital condyle. But in addition to this backward shifting of the hiatus nasopharyngeus there is another development of the palate peculiar to the *Crocodilia*. John Hunter (1861) described the "velum palati" of the crocodile. Owen (1866) wrote that "a velum palati is developed only in the *Crocodilia*", and Mivart (1873) speaks of the "transverse fold, or soft palate which hangs down in front of the posterior nares". Negus (1929), however, introduces a note of uncertainty, for he says "a velum of the palate is said to be present in the crocodiles, although it is scarcely to be seen in the specimens I have examined". In his Fig. 12 he names the "velum" of Hunter and Owen as "mouth flap" and it is to be presumed that he did not homologize this structure with the velum of the mammals.

The so-called velum palati of the crocodiles differs from the avian and mammalian structure known by the same name in that it is not perforated by the hiatus nasopharyngeus, but lies altogether anterior to it (see Fig. 2). John Hunter (1861) observed of the crocodilian velum that it arises "half an inch further forwards than (the posterior end of) the bony palate, so that it arises from the roof of the mouth". It seems quite obvious, therefore, that the crocodilian velum cannot be included in the definition of the true velum as that structure is recognized here. In no sense can it be considered as a continuity passing from the posterior extremity of the hard palate to form the roof of the pharynx and the dorsal wall of the oesophagus. The palatal flap of the crocodile contains no muscle between its reduplicated layers of palatal mucous membrane and no muscles pass to or from it to connect it with any adjacent structures. Its functional role is an entirely passive one, for in the action of closing the mouth

cavity from the pharynx, the "valve of the tongue" (John Hunter), which is constituted by the anterior margin of the hyoid, is thrust up in front of it by muscular action (see Fig. 3).

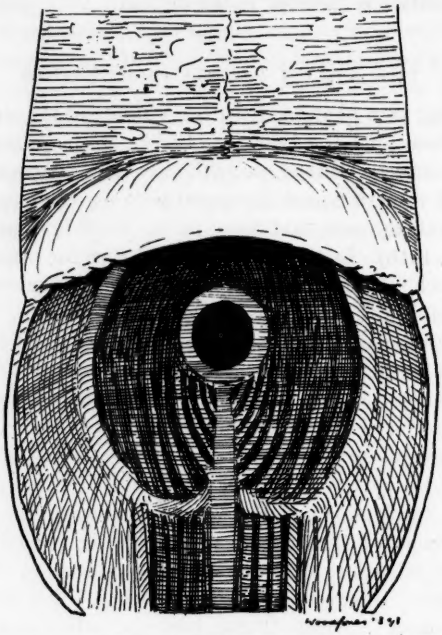

Fig. 2. Palate and dorsal wall of pharynx of *Crocodilus niloticus*. The palatal flap lies anterior to the hiatus nasopharyngeus and has no relation to it.

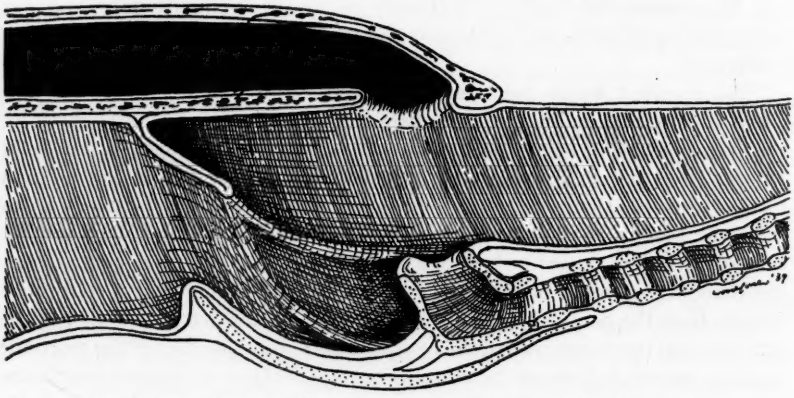

Fig. 3. Section of nasal chamber (black), palate, palatal flap, pharynx and larynx of *Crocodilus niloticus*.

Although this palatal flap of the *Crocodilia* was, presumably, homologized by Hunter, Owen, Cuvier, Mivart and others with the velum palati of mammals, it seems obvious that no such homology can be defended. In abandoning this incorrect homology—as undoubtedly it must be abandoned—one curious piece of evidence which might be raised in its support must be noted. The human anatomist, in modern text-book descriptive anatomy, has added to the confusion by claiming that the human velum forms a "septum between the mouth and the pharynx". This is exactly the role that the crocodilian palatal flap plays in the specialized economy of the crocodiles, but which, in fact, the true mammalian velum does not subserve.

There can be no doubt that the palatal flap is a specialized development in the crocodiles and that it subserves a very obvious and essential function that is demanded by the peculiar and specialized mode of life of these animals. It cannot be considered as being in any way a reptilian expression of any phase of development of the true velum palati.

THE VELUM PALATI IN BIRDS

Of the avian palate it may be said in general that although a vast amount of detailed work has been done upon the arrangement of the bones constituting the palate, the descriptive accounts of the roof of the mouth and pharynx in its entirety are by no means so detailed. Following the lead of Huxley in 1867, systematic ornithologists became habituated to the study of the bones entering into the formation of the avian palate as a basis of the well-known scheme of classification: but no comparable study of the soft parts of the palate accompanied this anatomical activity. In the year preceding that in which Huxley outlined his scheme of avian classification by reference to the structure of the bones of the palate, Owen (1866) had declared that "the posterior apertures of the nostrils being generally in the form of narrow fissures are undefended by a soft palate or uvula".

It must at once be admitted that if anything in any way resembling the human soft palate is looked for in the horny roof of the avian mouth, it is looked for in vain: but if by a velum palati we mean a continuity passing from the posterior end of the hard palate to form the roof of the pharynx and dorsal wall of the oesophagus, then it cannot be denied that such a structure exists in birds.

The opening of the nasal chambers into the roof of the mouth takes very different forms in the avian series and the details become further complicated by the varying relations taken by the Eustachian chamber: but in general it may be said that from the bony palate and around the nasal and Eustachian orifice, the roof of the mouth and pharynx—or palate—is continuous with the dorsal wall of the oesophagus, and this is the essential feature of the velum palati in its full development. In the Tubinares, in which the nasal chambers, the turbinates, and all the parts subserving the olfactory sense are particularly

well developed, an obvious velum exists. The orifice of the nasal chambers is ovoid in shape and it may, or may not, be continued forwards as a linear cleft in the mid-line of the hard palate. Around the orifice and extending backwards is a frill-like extension of the palate which passes back to the dorsal wall of the gullet. This velum shares in the somewhat horny character of the whole of the roof of the mouth, nevertheless it is without doubt the homologue of the soft palate such as is generally recognized in mammals. The velum is perforated in a typical manner by the orifice that leads into the nasal and Eustachian chambers: this is the true hiatus nasopharyngeus or rima nasalis of Dionis. It is of interest to note that the elevated tubercle, upon which the laryngeal orifice opens, is reciprocally adapted to the concavity of the rima nasalis. It is impossible to disregard the basal similarity of this condition to that seen in primitive mammals. The likeness to the condition present in *Ornithorhynchus* is particularly striking. The palate of *Prion turtur* is illustrated here (Fig. 4): elsewhere (1937), I have described and figured the palates of other members of the Tubinares, *Puffinus tenuirostris* and *Diomedea cauta*, which in all essential details are quite similar. Despite the assertion, made by Owen in 1866, that no homologue of the soft palate exists in birds, there were several accounts in the literature in which no doubt was left by the writer that he accepted the avian structures as being akin to the soft palate of mammals. In particular the work of Samuel Collins should be mentioned. In his *Systeme of Anatomy, etc.* (1685) he gives excellent figures and descriptions of the palates of several birds. Especially noteworthy are his figures and descriptions of the palate of the swan (Table 4, Fig. 1) in which he says that "the termination of the palate is made in two oblong glandulous processes", which extend behind the frilled edge that marks the posterior margin of the rima nasalis.

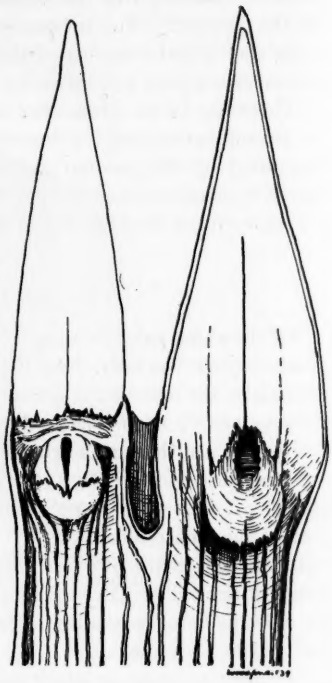

Fig. 4. Palate and dorsal wall of pharynx of *Prion turtur*. The pharynx has been opened along its left margin and the tongue and larynx reflected to the right.

It may be said that in birds the bony palate, which is the floor of the nasal chambers and the roof of the mouth, is continued backwards by a velum palati so as to join the dorsal wall of the pharynx and oesophagus and that this continuous velum is pierced by a hiatus nasopharyngeus or rima nasalis, beneath

which, on the floor of the mouth, behind the tongue, the opening of the larynx is seated. It must, therefore, be claimed that a true velum palati exists, in its whole continuity, in birds.

THE VELUM PALATI IN MAMMALS

In all primitive mammals, no matter if they be members of the *Ornithodelphia*, *Didelphia* or *Monodelphia*, the velum palati is in identical form. This primitive form is most readily appreciated by saying that the roof of the mouth is continuous with the roof of the pharynx and the dorsal wall of the oesophagus, which in primitive mammals are extended in nearly the same axis. This continuity between the roof of the mouth and the dorsal wall of the oesophagus is interrupted only by the aperture that permits the nasal chambers to communicate with the cavity of the pharynx. Beneath this aperture the orifice of the larynx projects behind the base of the tongue and through the aperture the orifice of the larynx may be thrust into and retained within the nasal chamber. In all primitive mammals the hiatus nasopharyngeus is surrounded by a sphincteric ring of muscle (m. palatopharyngeus) and it depends, almost certainly, upon the conditions that immediately preceded death whether the epiglottis and laryngeal orifice remain in the nasal chamber or are withdrawn from it.

The whole story of the old discussion of the intranarial epiglottis almost certainly depends upon the conditions prevailing at the time of death, for the thrusting up and withdrawal of the larynx through the velar aperture are active and vital muscular processes dictated by the needs of the moment. Just as a snake can herniate its laryngeal orifice from its mouth when engaged in swallowing something so large that it blocks up the whole of its oral cavity and pharynx, so can the primitive mammal herniate its laryngeal orifice into its nasal chambers and thereby establish a direct airway from its nostrils to its lungs. This subject is referred to again in considering the functional significance of the intranarial epiglottis.

The condition of the velum palati and the nasopharyngeal aperture is illustrated here in *Ornithorhynchus* (Fig. 5) as an example of the Monotremes, in *Dasycercus* (Figs. 6, 7) and *Dasyurus* (Fig. 8) among the Marsupials: in *Crocidura* (Fig. 10) representative of the Insectivora, *Pedetes* (Fig. 11) as a Rodent, and in *Lemur* (Fig. 12). But exactly comparable conditions are present in all mammals with the exception of the higher Haplorine Primates (*Pithecoidea*). Among the *Ornithodelphia*, both *Ornithorhynchus* and *Echidna* are typical of the condition described and figured: and the outstanding features of the horse, certain deer, and the cetaceans, have been frequently referred to in the literature. It may be accepted that the typical mammalian velum is the anterior portion of the dorsal pharyngeal wall, separated from the dorsal wall of the rest of the pharynx and oesophagus, in the mid-line only, by the hiatus nasopharyngeus, but continuous with them upon either side of the hiatus.

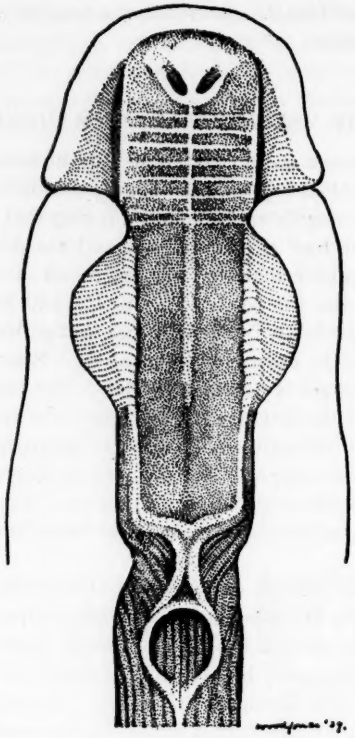

Fig. 5. Palate and dorsal wall of pharynx of *Ornithorhynchus*.

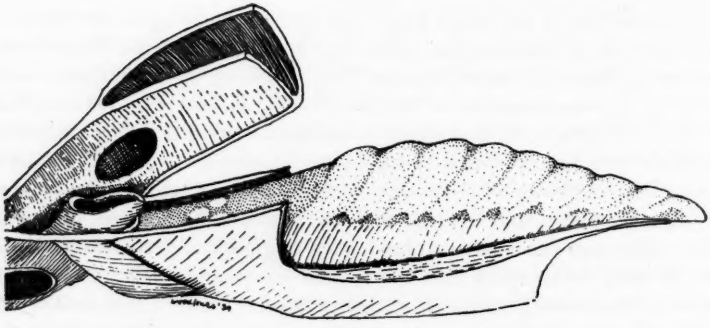

Fig. 6. Palate, hiatus, larynx and tongue of *Dasycercus*.

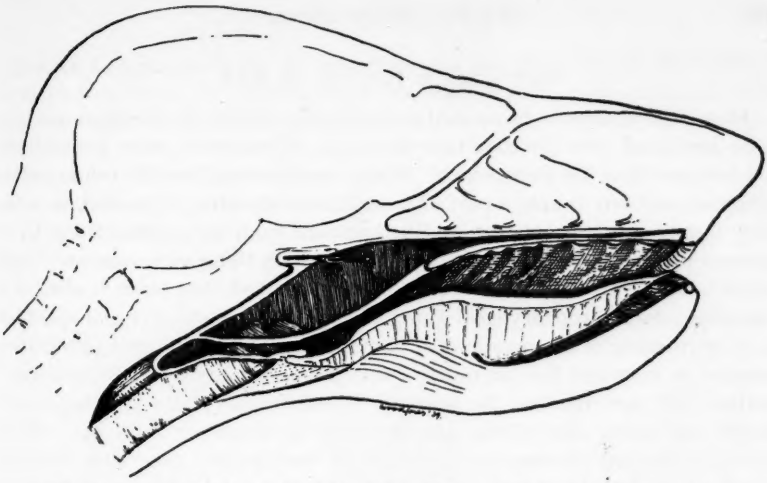

Fig. 7. Section, slightly to the right of the mid-line, of the head of *Dasycercus* to show the normal position of the palate and larynx.

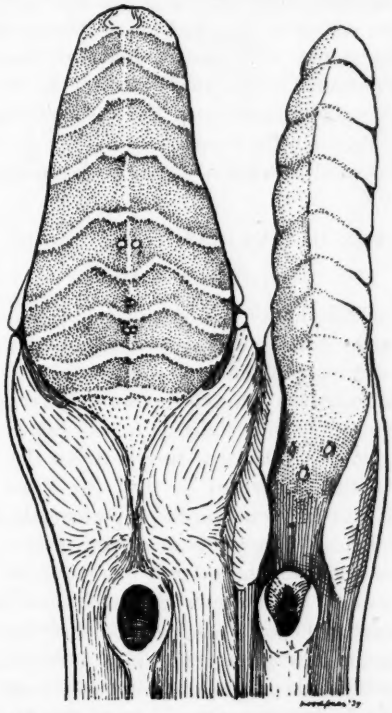

Fig. 8. Palate and dorsal wall of pharynx of *Dasyurus*. The pharynx has been opened along its right margin and the tongue and larynx reflected to the left.

THE VELUM PALATI IN MAN

How is the classical velum palati as described and defined in human anatomy to be correlated with the condition present in all mammals more generalized in this regard than the Pithecoidea? It is at once obvious that the velum palati of human anatomy is only a part of a continuous structure separated, in arbitrary fashion in nomenclature and description, from its continuations by a misconception of its true nature. There is no such thing as a separate "soft palate", with a free posterior margin in any mammal: but there is always a continuous dorsal oropharyngeal wall perforated by a nasopharyngeal aperture in every member of the mammalian phylum. The nasopharyngeal aperture is enlarged in man and the laryngeal orifice has ceased to occupy its primitive relation to it: nevertheless, the primitive dorsal oropharyngeal wall is present despite the undue size of the aperture that perforates it (Fig. 14). Quite obviously the posterior margin of the human "soft palate" marks the anterior boundary of the orifice; and equally obviously the palatopharyngeal folds or "posterior pillars" (as Dionis, Winslow, Monro, Trew and certain other human anatomists have recognized) constitute its lateral free borders (Fig. 15). But it does not seem to have occurred, even to Gustav Passavant, that the ridge on the posterior pharyngeal wall, now named after him, constitutes its posterior margin. Perhaps to Samuel Collins (1685) belongs the credit for having first illustrated the complete circumference of the nasopharyngeal hiatus in man. The figures of the condition in the human foetus given in this paper should be compared with Zuckerkandl's excellent figure of the same parts in the human adult (1904).

THE HIATUS NASOPHARYNGEUS

(*a*) *Anterior margin*

One well-known distinction of the human velum is its richness in glands that pour their secretions into the buccal cavity. In particular these glands are aggregated at the hinder end of the human soft palate, or at the anterior margin of the hiatus nasopharyngeus. When Owen observed that in birds the apertures of the nasal chambers were "undefended by a soft palate or uvula", he overlooked the similarity in glandular structure between the velum of birds and mammals. Samuel Collins described in 1685 the "two oblong glandulous processes" in the palate of the swan and this is perhaps the first reference in the literature of the well-known glandulae sphenopterygoidae in birds. In all the mammals that I have examined the anterior margin of the hiatus is prominent and tumescent and usually this tumescence is continued forwards in the mid-line of the velum towards the hard palate. This is particularly conspicuous in *Crocidura* (Fig. 10) but in most forms it is evident in some degree. The swelling is also particularly well marked in the mid-line of the anterior margin of the hiatus itself. In order to ascertain the nature of this tumescence, the hiatus and its immediate surroundings were excised in a series of mammals (*Homo*

(foetalis), *Lemur*, *Crocidura*, *Pedetes*, *Dasyurus* and *Dasycercus*). The excised portions were flattened between two slides, cleared with xylol and mounted in Canada balsam. Under the microscope the tumescence of the anterior margin

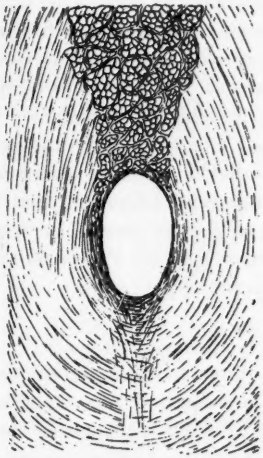

Fig. 9. Palate and dorsal wall of pharynx of *Dasyurus*, prepared as described in text, to show glandular tissue and sphincteric fibres of m. palatopharyngeus.

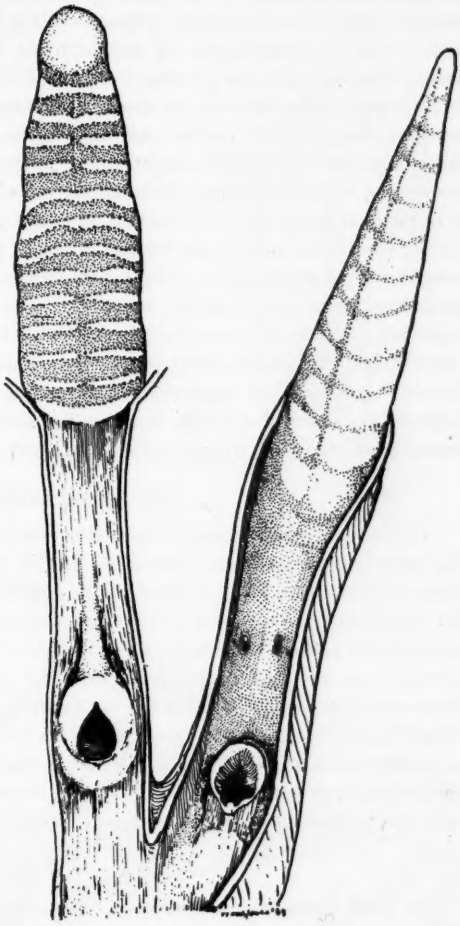

Fig. 10. Palate and dorsal wall of pharynx of *Crocidura*. The tongue and larynx are reflected to the left.

of the hiatus was clearly revealed as being due to glandular masses in the thickness of the velum. These glandular masses conformed to a general type. In the more anterior part of the velum the main masses tended to be bilateral, but in the posterior part, at the anterior margin of the hiatus nasopharyngeus, they became confluent in the mid-line and extended some distance around the

anterior margin, causing the elevation seen on the surface. It appears to be this mid-line aggregation of glandular tissue, which in some animals (see Fig. 11) causes a median thickening in the anterior margin of the hiatus, that ultimately culminates in the development of a uvula. The development of a definite uvula is a question of degree and it is by no means a simple matter to define exactly what degree of development warrants the assertion that an animal does or does not possess a uvula. In *Pedetes* the aggregation of glandular tissue in the mid-line produces a definite prominence or tubercle on the anterior margin of the hiatus nasopharyngeus. This median tubercle, which is present in many mammals, might not be generally accepted as being a declared uvula, but at least it appears to be the anatomical basis from which a definitive uvula is developed in certain forms. It might be suggested that the more completely the hiatus is occupied by the orifice of the larynx, the less would be the irregularity in the margin of the hiatus caused by the mid-line aggregation of glandular tissue. Conversely, we might expect that when the larynx is permanently withdrawn from the hiatus, this mid-line development of glandular tissue might culminate in the production of a pendulous prolongation thereby developing into what is generally recognized as a uvula. In general such an expectation is fulfilled in the mammalian series, for it is in the Pithecoid Primates alone that the uvula is generally accepted as being present.

(*b*) *Lateral margins*

In the cleared specimens prepared as described above, it was apparent that the prominent lateral margins of the hiatus were composed of mucous membrane folded around well-defined strands of muscular fibres which, arising from the bony palate, continued around the margins of the hiatus to meet behind it and join the posterior (dorsal) wall of the pharynx. These fibres are undoubtedly those of the *m. palatopharyngeus* and their posterior merging into the dorsal pharyngeal wall is discussed in the next section. In such marsupials as *Dasyurus* (Fig. 9) and *Dasycercus* the meeting of the fibres behind the hiatus constitutes a definite median elevation on the dorsal wall of the pharynx. In most generalized mammals the muscles of the two lateral margins meet as a sphincteric ring within the encircling pharyngeal musculature.

(*c*) *Posterior margin*

In 1869 Passavant described a "cross-roll" on the posterior wall of the pharynx at the level of the soft palate. This ridge he considered as a normal feature, produced by efforts to close the hiatus nasopharyngeus in the ordinary phonation of all vocal sounds other than those having a nasal tone. He particularly stressed its high development and the ease with which it could be demonstrated in cases of cleft palate where the functional need for its presence was peculiarly necessary. As for its causation, he realized that it was produced by muscular fibres encircling the back of the pharynx, but he did not share Luschka's opinion that the palatopharyngeus was of great importance in this

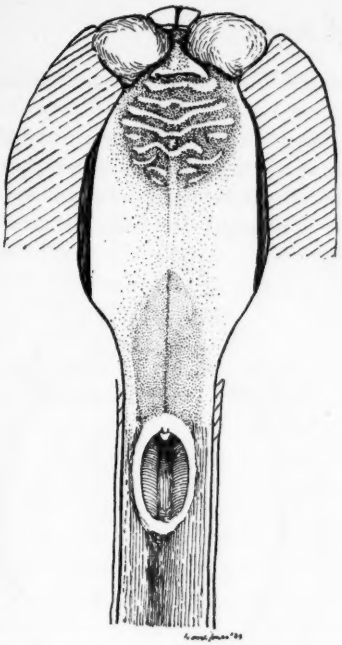

Fig. 11. Palate and dorsal wall of pharynx of a neonatal specimen of *Pedetes*.

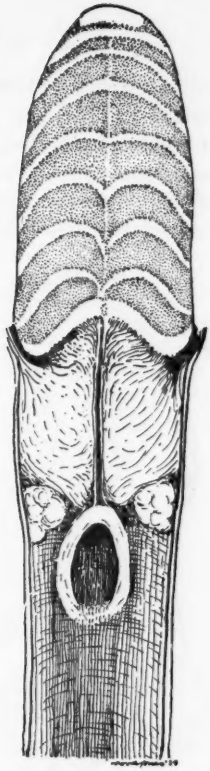

Fig. 12. Palate and dorsal wall of pharynx of *Lemur*.

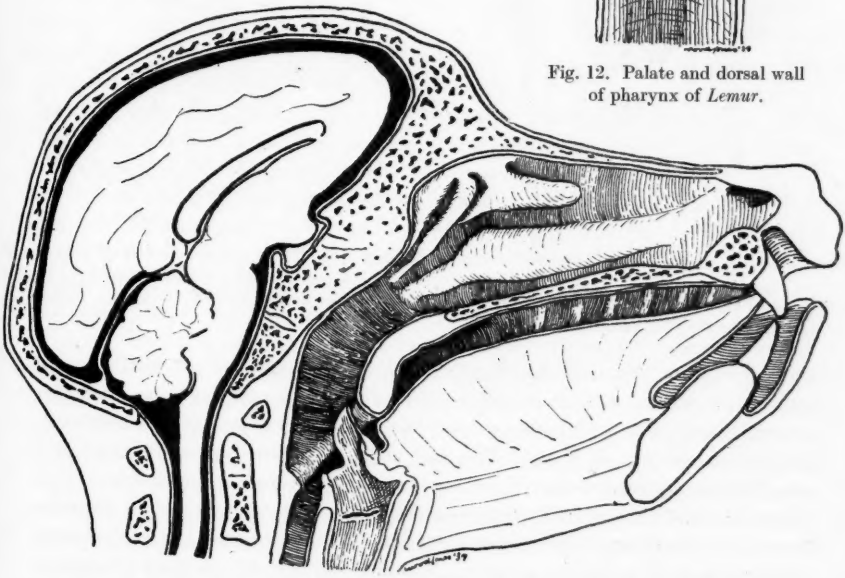

Fig. 13. Section of the head of a baboon to show relation of the larynx to the hiatus nasopharyngeus, the posterior margin of which is "Passavant's bar".

regard and considered that it was "an isolated function of the superior constrictor muscle".

It would appear that, with the exception of Jonnesco's work, but little attention was paid to Passavant's bar from an anatomical point of view until Wardhill revived the subject in 1928 and Whillis published his description of the musculature in 1930. In this last paper it is stated that "the presence of

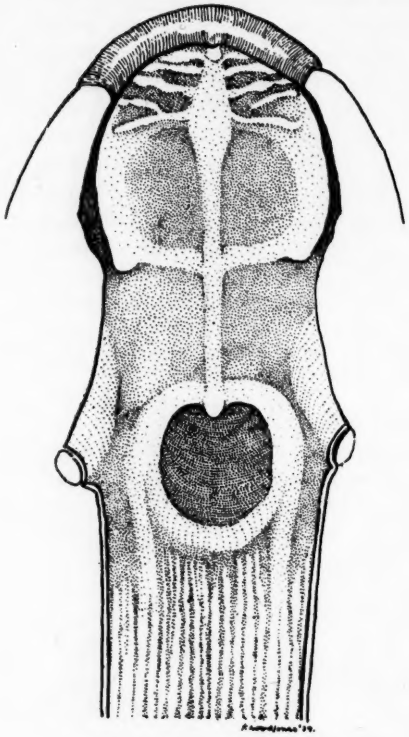

Fig. 14. Palate and dorsal wall of pharynx of a human embryo of 74 mm. R.V. length.

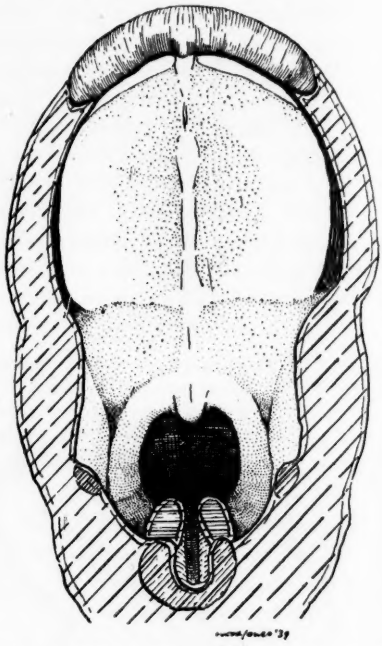

Fig. 15. Palate and dorsal wall of pharynx of a human embryo of 100 mm. R.V. length. The dorsal wall of the larynx remains *in situ*.

the ridge led us (Wardhill and Whillis), in discussing the problem, to the conclusion that there must be some muscular mechanism producing it, and that the muscle fibres must run transversely. No mention was made in Passavant's original paper of such fibres." This last statement is a somewhat remarkable one. Whillis concluded that the muscle responsible for the production of the ridge was a definite slip of the superior constrictor muscle and herein, of course, he restated Passavant's original conclusion. This muscular slip he named as the *palatopharyngeal sphincter*, a designation reminiscent of Luschka's (1862) *constrictor isthmi pharyngo-nasalis*. The nature of these fibres can only be made

clear by a review of the disposition of the palato-pharyngeus muscle of human anatomy. The confusion that has arisen with regard to the actual muscle that pulls the posterior wall of the pharynx forwards "at the level of the base of the uvula" is due to the very varying ideas that have been held by anatomists concerning the muscles that, attached to the palate, pass to other parts of the mouth and pharynx. In brief, there are three separate muscles that radiate to the tongue, the larynx and the posterior pharyngeal wall respectively. The fibres passing from the palate to the tongue—the *m. palatoglossus*—may be dismissed from the discussion since no great discrepancy in their description has been conspicuous in the literature. In generalized mammalian types there is, posterior to the m. palatoglossus, a separate muscle passing from the palate to the larynx. This is the m. thyrostaphylinus or m. thyropalatinus of Winslow, Cloquet, Jonnesco and other anatomists. In the interests of uniform nomenclature this muscle is here termed *m. palatothyroideus*. The third muscle, more or less confluent with the others at its attachment to the palate, passes around the hiatus nasopharyngeus and embraces the pharynx "at the level of the base of the uvula". This muscle is the m. pharyngostaphylini of Winslow, the m. pharyngostaphylinus plus the m. peristaphylopharyngeus of Cloquet. It was more definitely named by Luschka as m. pharyngopalatinus seu m. constrictor isthmi pharyngonasalis. For this muscle the best name is that of Albinus—*m. palatopharyngeus*. Unfortunately it has been the custom of most modern anatomists to combine the laryngeal and pharyngeal muscles under the name given by Albinus to the pharyngeal portion only. It matters not that this muscle is usually divided in modern descriptive anatomy into dorsal and ventral or medial and lateral parts, the correct conception of two separate morphological and functional elements is lost sight of by the employment of our present terminology. In the generalized mammalian type these three muscles, the one passing from the palate to the tongue, the second from the palate to the larynx and the third surrounding the hiatus nasopharyngeus, are not widely sundered even at their insertions, since the tongue, the larynx and the posterior pharyngeal wall are situated at nearly the same level. In man the parts have become readjusted to different levels. The *m. palatoglossus* remains in a more or less primitive condition and there is no problem concerning its identity in human or generalized mammalian anatomy. The *m. palatothyroideus* has undergone a marked change in man consequent on the sinking of the level of the larynx below the velum. The *m. palatopharyngeus* has, to a certain extent, become involved in this change, for its lower fibres are pulled down the posterior pharyngeal wall in the wake of the descending larynx. Nevertheless its upper fibres retain their primitive position and continue to carry out their original sphincteric function. This fact was recognized by many earlier anatomists, and John Innes (1804) especially notes that the fibres of the palatopharyngeus "run backwards to the *top* and lateral parts of the pharynx". There is no need to discuss those fibres that passing above the level of the m. palatopharyngeus have been described as a separate muscle—the *m. salpingo-*

pharyngeus. By some anatomists they have been regarded as part of the m. palatopharyngeus, and by some as part of the superior constrictor of the pharynx. It matters little to which morphological plane they are assigned; but probably the clearest definition of their morphology is that of Macalister (1889). As a consequence of confusing the m. palatothyroideus and the m. palatopharyngeus anatomists have come to assume that the fibres of this latter muscle are disposed almost vertically in man. This being so there has been a tendency to assign to the stratum of the superior constrictor of the pharynx those upper fibres of the palatopharyngeus that encircle the pharynx in a nearly horizontal axis. It was these fibres that, in 1869, Passavant assigned to the superior constrictor and which were rightly held by him to be responsible for the production of the sphincteric action of the hiatus nasopharyngeus. In 1879 Sappey drew attention to these fibres and described them as a separate muscle—his *m. occipitostaphylinus*—but considered them to be the highest portion of the superior constrictor of the pharynx, although he noted their common origin with the palatopharyngeus. It is these same fibres that were redescribed by Whillis in 1930 as part of the superior constrictor and to which he gave the name palatopharyngeal sphincter. More recently (unpublished work, 1939) R. H. Townshend has re-examined the whole question and has determined that Passavant's bar, which he examined exhaustively in a patient with cleft palate, is undoubtedly due to this sheet of muscle and that there is no doubt whatever that the muscle is the upper part of the palatopharyngeus and is no part of the superior constrictor. Such a finding justifies Luschka's name, constrictor isthmi pharyngo-nasalis, as a designation for the upper fibres of the palatopharyngeus. It also confirms Jonnesco's account of the action of the palatopharyngeus, when he says: "Cette occlusion due, d'après Passavant, uniquement à la contraction des constricteurs supérieurs, est produite surtout par l'action des muscles pharyngo-staphylinus, dont les constricteurs ne sont que les auxiliaires" (1912, p. 170). It is evident that the human palatopharyngeus has not departed widely either in morphological type or in function from the condition prevailing in the more generalized mammalian types.

THE FUNCTIONAL IMPLICATIONS OF THE PRIMITIVE MAMMALIAN VELUM

Dr R. L. Bowles, in his paper "Observations upon the mammalian pharynx, with especial reference to the epiglottis" (1889), was much concerned with the inconstancy of his findings and those of his assistant on the actual position of the larynx in the young pig. "My assistant, Mr Stainer, has introduced his hand into the pharynx of many pigs of six months and upwards and has always found the velum to lie anteriorly to (below) the epiglottis and firmly clasping its root. This being so, I was not a little surprised to find that in one animal (said to be five months old) the epiglottis lay much more within the buccal cavity than I should have suspected." This last finding was made by the dissection of the dead animal and not, like Stainer's observations, by examination

of the living. All the variations in position of the larynx with relation to the velum detected by Bowles were looked upon by him as due to age changes and to relative growth; and he regarded the position attained at any particular age as a definitive one. Howes, however, realized that age changes alone could not account for the varying conditions met with and he appreciated the fact that the variations found post-mortem might be due to displacement of the larynx by traction and manipulation during dissection. The fact that there was no sort of abnormality of the larynx or velum in the two rabbits in which he found the epiglottis intranarial in position on dissection justified such a supposition. But it is quite certain that the manipulation of the parts during dissection does not wholly account for the varying positions found, for the examination of several specimens of any species will reveal wide variations even when the utmost precautions are taken against disturbance of the parts. It is far more likely that in the act of death the larynx is commonly withdrawn from the nasal chambers in most animals having an intranarial epiglottis: it is only occasionally that an animal dies with its larynx in its nasal chambers and this event only happens with any frequency in those animals in which the larynx is particularly tightly held within the sphincteric ring of the hiatus nasopharyngeus. In *Dasycercus* I have found the larynx in the nasal chambers in the majority of specimens examined: but in *Dasyurus* and other marsupials it is commonly buccal in position in the dead animal. It is obvious and it is generally recognized that the intranarial position of the larynx is a primitive mammalian mechanism for securing an airway direct from the anterior nares, to the nasal chambers, larynx, trachea and lungs. From this direct air passage the buccal cavity and pharynx are altogether shut off. Food, passed from the mouth and via the pharynx to the oesophagus, is guided upon either side of the larynx through bilateral channels, the remnants of which are represented by the piriform fossae in man. Although the intranarial position of the glottis admits to perfection the functions of olfactation and respiration, it is apparently incompatible with vocalization. In such mammals as are capable of producing vocal sounds, the glottis must be withdrawn from the nasal chambers so as to take up a buccal position during vocalization. No doubt the relaxation of the sphincteric muscle of the hiatus nasopharyngeus, combined with the action of muscles passing to the larynx, is concerned in this movement of withdrawal: but other factors are certainly involved. The actual site of the hiatus nasopharyngeus varies rather widely in different species of primitive mammals, being in some considerably farther back than in others. But apparently its position depends entirely upon the build of the skull, for it would seem to be a general rule that it lies between the temporomandibular and the occipito-atlantal articulations. It is obvious, therefore, that any extension of the head on the occipito-atlantal articulation will tend to withdraw the glottis from the nasal chambers and the completion of the withdrawal will be effected by opening the mouth (see Fig. 13). It will be recalled that most mammals that are capable of producing vocal sounds have, as a preliminary to any vocal effort, a general

movement of extending the head on the neck. This raising of the head appears to be regarded in a vague sort of way as an aid to the dissemination of the voice: but I imagine that it is a necessary preliminary in animals having an intranarial epiglottis in order to insure a buccal position for the larynx. The opening of the mouth completes the movement and the animal is free to produce its vocal sound. A dog may growl with a flexed head: but I doubt if it can bark or especially howl to much effect without some degree of extension of the head on the neck. It is noteworthy that the primitive marsupials, in which the larynx is most completely intranarial, are for the most part incapable of producing vocal sounds. It is also to be remembered that man, in whom the larynx is most completely and permanently extranarial, is the most continuously vocal of all mammals.

NOTE ON THE WORK OF RÜCKERT

Rückert published his monograph *Der Pharynx als Sprach- und Schluckapparat* in 1882, and although it gives an exhaustive account of the palate of man and lower animals, it appears to have been very generally overlooked by later writers. For the use of the copy that I have been able to consult I am indebted to the Zoological Library of the University of Bristol. Rückert was apparently unacquainted with the work of Passavant, for he describes the ridge to which Passavant called attention and names it indifferently as the "crista" or the "torus" of the dorsal pharyngeal wall. In describing the velum he mentions the "arcus", which surrounds what is here termed the hiatus nasopharyngeus, and says "only the anterior part of this ring as far as it belongs to the velum palatinum contains muscular fibres, the posterior part consists only of two mucous membrane layers firmly connected with each other". It is evident that he pictured his "crista" as being merely a mucous membrane ridge. Later on, however, in speaking of the crista of lower mammals he observes "This connexion is not a casual one, as the fibres of the m. pharyngopalati continue to the posterior wall in order to unite in the middle line."

Rückert was acquainted with the work of Luschka on the human velum: but it would seem that he did not realize that his "arcus pharyngo-palatinus", which in man clothes the m. palatothyroideus, had been separated (by the fall of the larynx) from the true arcus pharyngopalatinus (such as he describes in lower mammals) which contains the m. palatopharyngeus. Rückert failed to recognize the muscular nature of the crista pharyngea or pharyngeal torus that had been so well described by Passavant thirteen years previously: but all other characters of the true velum and the limits of the pharynx he described in masterly fashion.

SUMMARY AND CONCLUSIONS

1. The velum palati is not confined to mammals, being present, in comparable form, in birds. The so-called velum of the *Crocodilia*, however, cannot be homologized with the mammalian velum.

2. The primitive condition of the velum in mammals consists in a complete continuity from the roof of the mouth to the dorsal wall of the pharynx.

3. This continuity is broken only by the presence of the hiatus nasopharyngeus by which the rima glottidis establishes continuity with the nasal chambers.

4. It is violating the elementary principles of anatomical nomenclature to extend the term "pharynx" to any part of the nasal chambers: and therefore the term "pharyngeal isthmus" for the hiatus nasopharyngeus is misleading and should be deleted from nomenclature.

5. The definition of the "soft palate" as "a movable fold, suspended from the posterior border of the hard palate, and forming an incomplete septum *between the mouth and pharynx*" (as in Gray, 1938, p. 1263, and most modern text-books of human anatomy) is absurd. The velum in no mammal separates the mouth from the pharynx: but it always separates the pharynx from the nasal chambers.

6. The m. palatopharyngeus of modern text-books of human anatomy is composed of two separate muscles, confused in modern terminology—the m. palatothyroideus and the true m. palatopharyngeus.

7. The true m. palatopharyngeus is the muscle that has been mistaken by many human anatomists for the upper fibres of the superior constrictor of the pharynx.

8. The true m. palatopharyngeus is the sphincter of the hiatus nasopharyngeus and is responsible, in man, for what is known as "Passavant's bar".

9. The anterior margin of the hiatus nasopharyngeus is glandular in all mammals. In mammals in which the rima glottidis is permanently, or even frequently, withdrawn from the hiatus this glandular tissue is aggregated in a median tubercle culminating in the development of the uvula.

10. The position of the rima glottidis in relation to the velum is commonly altered by the conditions prevailing at death.

11. In such animals as are capable of phonation and have the rima glottidis habitually in the nasal chambers, it must be withdrawn (by extension of the head, opening of the mouth or by other means as auxiliaries) during phonation.

12. The condition of the Pithecoidea, and especially in *Homo*, is characterized by an enlargement of the hiatus nasopharyngeus and, especially in *Homo*, by the permanent withdrawal of the rima glottidis from the nasal chambers. In *Homo* this change in structural relations is definitely related to the constant vocalization characteristic of man.

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AN EXPERIMENTAL STUDY OF THE MORPHOGENESIS OF INTERSEXUALITY

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The only experimental investigations of mammalian intersexuality prior to 1937 appear to be those of Baker (1925*a*) and of Krediet (1930*a*), who commented on the retrogressive changes in the uterine horns of castrated intersexual pigs. Since 1937 the problem has attracted more attention, and has been investigated experimentally in two ways: (1) by the injection of sex hormones into intersexual animals, and (2) by the injection of sex hormones, with a view to influencing sexual development, either directly into guinea-pig foetuses *in utero* or into pregnant female rats and mice.

The first type of investigation has been carried out by Wells (1937), who injected pregnant mare serum into an intersexual ground squirrel (*Citellus tridecemlineatus*). As a result of these injections spermatogenesis and luteinization were stimulated in the ovotestes, and growth occurred in all the male and female accessory reproductive organs, this finding suggesting that the gonadotrophic hormone had stimulated the gonads to produce both androgenic and oestrogenic hormone. The second type of investigation has been carried out by several workers. Thus, female guinea-pig embryos have been altered in a male direction by being injected *in utero* with testosterone propionate (Dantchakoff, 1937*a*, *b*, *c*, *d*; 1938*a*, *b*; 1939*a*, *b*, *c*). The external genitalia became masculinized, and vasa deferentia, seminal vesicles and prostates developed. These organs existed side by side with female reproductive organs which were normal except for the opening of the vagina, as a rule, into the urethra. Similar effects have been produced in rats and mice by the injection of crystalline androgenic hormone into pregnant females (Greene & Ivy, 1937; Greene *et al.* 1938*b*; Raynaud, 1937*a*, *b*, 1938*a–h*; Hamilton & Gardner, 1937; Hamilton & Wolfe, 1938). The female genitalia of the foetuses became altered in a masculine direction, while at the same time male accessory organs developed. The uterus, the oviducts, and the upper part of the vagina persisted, the fact that the lower part of the vagina did not persist suggesting that its embryological origin is not the same as that of the upper part. Somewhat similar results had previously been obtained by injecting testicular extracts into newborn rats (Wiesner, 1935), while contrary results have recently been reported in opossum pouch young by Moore (1939) (e.g. stimulation of Müllerian duct by androgens).

The reverse changes have also been observed. Greene *et al.* (1938*a*), who injected oestrogenic hormone into pregnant rats, found that the male offspring

had smaller male accessory reproductive organs than is normal, and also vaginae and nipples similar to those of the newborn female of the species. Reduction of the male accessory organs was not, however, observed by Wiesner (1935) when newborn rats were injected with oestrogenic hormone. On the other hand, Hain (1935*a*, *b*, *c*, 1936) found that both the female foetuses of rats injected during pregnancy, and the female young of rats which are injected during the early puerperium, exhibit a partial hypospadias—an abnormality which is associated with intersexuality. In Hain's experiments, it may be noted, oestrogenic treatment had no effect on male foetuses or male young, which, like the female young, were also unaffected by androgenic treatment. More recently Raynaud (1939*a–f*) has made a close study of the effects which oestrogenic hormone, when injected into pregnant mice, has upon male foetuses. In addition to the changes described by Greene *et al.*, he reports the persistence of the Müllerian ducts and metaplastic changes in the epithelium of the urogenital sinus.

These experimental observations throw considerable light on the morphogenesis of the accessory reproductive organs. It should, however, be noted that no observer has as yet reported the experimental induction by means of sex hormones of all the changes which are encountered in natural intersexuals. Until this has been done the investigator of intersexuality cannot afford to neglect the study of natural sex-intergrade animals.

The present paper is concerned with the experimental investigation of an intersexual pig (*Sus scrofa*). Observations were made on the animal's gonads, uterus and vas deferens (1) in their normal intersexual condition, (2) after 3 months' treatment with androgenic hormone, (3) after 3 months' treatment with oestrogenic hormone, and (4) 4 months after complete removal of the gonads. The results of this study, as is shown in the conclusion to the paper, add significantly to the data which have to be taken into account in interpretations of intersexuality.

MATERIAL

The animal used in the present investigation was a "Large White" pedigree pig supplied by Mr G. Garceau of Padstow, Cornwall, through the National Pig Breeders' Association. Mr Garceau reported that the animal exhibited male behaviour when it was 3 months old; it would ride sows much more actively than would a normal gilt. On the other hand, it was plain from the conformation of its external genitalia that the animal was an intersex.

It had a penile tuft in the region where the penis ends in the normal male, and a small genital eminence. Both testes were descended, and were palpable in definite scrotal sacs, which lay in front of the eminence. Dorsal to the latter there was a vulva of the intersex type (see below). Unlike the normal sow, urination took place in an upward stream, sometimes in a series of jets.

Mr Garceau had bred four other intersexes, all of which were inbred. As it has been suggested that the intersexual condition is inherited through the

boar (Baker, 1925*a*), it may be worth noting that the male parent of the animal under consideration sired two other intersexes by another, but related, sow, and that the mating of two of its normal offspring produced two more intersexes. It became infertile at 4 years of age.

THE REPRODUCTIVE ORGANS

Although the reproductive organs of the animal were not examined thoroughly until its autopsy, it is advisable to describe them before the experimental observations are recorded. The measurements given below were taken after fixation of the specimen in Bouin's fluid.

The external genitalia appeared to be similar to those of the intersexual pig no. 1 figured by Baker (1925*a*). The anus was normal. It was separated by a very short perinaeum from the relatively large and crescentic opening of the urogenital sinus. This opening, which in normal sows is a sagitally disposed cleft situated just dorsal to the summit of the genital eminence, was bounded on each side by two labia, about 5 mm. thick, which passed forwards into either side of the eminence, whose projection into the sinus was responsible for the latter's semi-lunar opening.

In normal sows the clitoris (which in three control specimens was about 2 cm. long and 2–3 mm. in diameter) is held in the ventral wall of the urogenital sinus by two lateral folds of tissue which pass into the undersurface of the genital eminence. A narrow, somewhat crescentic, tubular recess is thus formed between the clitoris and the undersurface of the eminence, which, when pulled forwards, exposes the tip of the clitoris. In the experimental intersexual animal the clitoris had the same relations, but was very much enlarged, being 6 cm. long and 1 cm. in diameter. It was formed by the union of two corpora cavernosa, which diverged at its root into two normally disposed crura. Its surface was covered by closely set small papillae, and its end was conical.

The structure which has been referred to as the genital eminence, and which is called by Baker "ventral labial commissure", presumably represents the greater part of the genital tubercle of the embryo. Alternatively it may represent a special development, like the mons veneris (Spaulding, 1921). It was attached ventrally by a base 4 cm. wide, and its dorsally directed free and narrower end, the tip of which was pointed, covered the opening of the urogenital sinus and the tip of the clitoris.

The urogenital sinus, into the cranial end of which the urethra and the vagina opened, was about 10 cm. long, as it also was in a normal sow which was examined as a control. Its lining epithelium mostly consisted of low stratified cells (Fig. 22), and a diffuse gland, similar in structure to prostatic tissue, was present in its muscular coat (Fig. 18). The vagina was capacious and 16 cm. long (in a control specimen it was 24 cm. long). It was lined by one or two layers of cuboidal or columnar cells, and numerous glandular crypts opened into it. These crypts, again, formed the collecting ducts of numerous

tubular glands, which were lined by a single row of regularly disposed columnar cells whose nuclei filled the basal halves of the cells (Fig. 23).

It should be noted that these histological observations refer to the animal 4 months after its gonads were removed.

Externally, the corpus uteri, which was not sharply demarcated from the vagina, was 12·5 cm. long, but the lumina of the cornua remained separate for 3 cm. within the body of the uterus. The uterine horns, which appeared to be of normal length, passed out of the abdominal cavity into scrotal sacs, to become attached to the gonads. Their termination is described below.

Histological sections of several regions of the gonads (27 blocks were cut) failed to disclose any ovarian tissue, and the gonads, as noted below, consisted entirely of degenerate seminiferous tubules and interstitial tissue (Fig. 24). The epididymis of each side was large, the tail especially so, but close dissection and histological examination of several regions failed to reveal any vasa efferentia actually penetrating into the gonad. The vas deferens took origin in the usual manner from the tail of the epididymis, where the epididymal tubules were much more distended than either in the head or body of the organ.

In addition to the epididymis, a large, fatty, vascular and lobulated structure was attached to each testis. The rounded end of this organ was in relation to the head of the epididymis, while its attached and flattened base was applied to the tunica albuginea under cover of the body of the epididymis. On section, this organ was seen to consist mostly of coiled arteries whose diameter varied between about 1 and 2 mm. Smaller arteries, veins and lymphatics were also present. Presumably this structure represented the testicular part of the pampiniform plexus.

No Fallopian tube was present, and the uterus ended blindly on both sides in relation to the tail of the epididymis, which it reached by passing, with the vas deferens, down the medial surface of the body of the epididymis.

The vasa deferentia entered the abdominal cavity with the uterine cornua, and coursed medially in the broad ligament, becoming somewhat less prominent as they approached the body of the uterus. They then passed down the lateral margins of the ventral surface of the corpus uteri, embedded in the superficial fibres of the myometrium (Fig. 20), and ended by opening on each side into the apex of a vesicular gland which reached cranially 1·5 cm. above the os uteri.

The vesicular glands were paired lobulated and flattened structures which reached from 1·5 cm. above the level of the os uteri as far caudally as 2·5 cm. from the cranial limit of the urogenital sinus (Fig. 25). They were applied on each side to the muscular wall of the vagina, and the lobules became sparse and thin in the distal half of the gland. Each opened caudally into a duct of very narrow calibre, which ended blindly in the submucosa of the vagina immediately above the opening of the latter into the urogenital sinus (Fig. 23).

The vesicular gland in the normal boar is described as an organ whose main duct joins the vas deferens to form a common ejaculatory duct which opens on a small colliculus seminalis[1] (Disselhorst, 1904). The arrangement of the vesicular glands in our intersexual animal must thus be regarded as abnormal, especially in so far as they constitute not true seminal vesicles but ampullary glands of the vas deferens—glands which the normal boar does not possess. Baker does not refer to the relation of the seminal vesicles and vasa deferentia in the specimens of intersexual pig which he describes. Crew (1923) does, and he writes that "the vasa deferentia...passed through the substance of the seminal vesicles to enter the urethra". His Fig. 1 is a close representation of the condition observed macroscopically in our specimen. That the vasa deferentia in the latter did not pass through, but became, the "seminal vesicles" was established definitely by examination of serial sagittal sections of the junction of the two (Fig. 21).

A prostate was also present in our intersexual pig. It was a small compact organ situated in the angle between the urethra and the vagina (Fig. 18). No obvious prostatic tissue was present dorsal to the cranial part of the urogenital sinus, but histological section of the wall of the sinus in this region disclosed glandular tissue very similar to the prostate.

No bulbo-urethral glands were found, and it may be noted that these glands are not possessed by sows.

EXPERIMENTAL TREATMENT

First operation (3 February 1937)

When the animal was $6\frac{1}{2}$ months old, and before it had shown any signs of oestrus, a laparotomy was performed under ether anaesthesia, and a section of the right uterine horn, 10 cm. long, and of the vas deferens in relation to it, was excised. The medial end of the piece of uterus removed was about 5 cm. from the junction of the two horns, and its mean diameter was 1·2 cm. That of the vas deferens was 2·2 mm. The divided ends of the cornu and vas were ligatured, and the laparotomy wound was closed by means of three layers of catgut sutures.

A second incision was made into the scrotal sac, and a segment of the left testis was removed. The incisions in the testis and scrotum were also closed by means of catgut. The tissues removed were fixed in Bouin's fluid, a piece of testis also being fixed in Helly and formol.

Both operative sites healed quickly and by first intention.

The animal was kept under close observation during the 3 months intervening between 3 February and 10 May, but vaginal and vulval smears were not made, since it is stated that the oestrous cycle of the sow cannot be satisfactorily followed by this method of investigation. From behavioural

[1] According to Owen (1868), the duct of the seminal vesicle and of the vas deferens open independently into the urethra.

signs the animal appeared to be in oestrus on 19 February, 25 March and 18 April 1937 (i.e. at intervals of 35 and 24 days respectively). In the normal sow oestrus recurs approximately every 21 days; the fact that oestrous periods occur in intersexual pigs has been noted by Baker (1925*a*).

Injections of male hormone. During the 2½ months intervening between 10 May and 27 July 1937, the animal was injected every 3 or 4 days with a solution of testosterone propionate in oil. The injections were made intramuscularly, and a total of 1·05 g. was administered.

Oestrous behaviour was observed only during the first week of injections (19 May 1937).

During this phase of the experiment the animal was growing rapidly, and growth of its tusks and clitoris was observed. Whether or not these changes were part of general somatic growth, or whether they were stimulated by the injections of male hormone, could not of course be determined.

Second operation (4 August 1937)

Twelve days after the last injection of testosterone propionate, specimens of the right uterine horn, Gartner's duct and testis were once again removed under ether anaesthesia. Access to all tissues was obtained through an incision in the right scrotal sac. The wound was sutured, and healed rapidly as before.

The piece of uterus removed was about 10 cm. long, and its lateral end was 12 cm. from the termination of the right cornu on the testis. The uterine horn appeared smaller than on the occasion of the first laparotomy, its mean diameter being 7 mm. Fluid was present in its lumen.

Gartner's duct, a segment of which was also removed, was larger than it was at the time of the first operation, its mean diameter being 4·5 mm. A piece of the right testis was also removed.

Apart from a piece of testis which was placed in Helly and formol, all tissues were fixed, as before, in Bouin's fluid.

Injections of oestrogenic hormone. Between 19 August (a fortnight after the second operation) and 2 November 1937, the animal was injected intramuscularly, usually every 2 or 3 days, with a solution of oestradiol benzoate in oil. A total of 310 mg. was injected. During this phase of the experiment symptoms of restlessness were frequently observed, and the clitoris appeared to decrease in size. The animal continued growing, and there was no regression in the size of its tusks.

Third operation (8 November 1937)

Six days after the last injection of oestradiol benzoate, both testes with the attached parts of the uterine cornua and Gartner's ducts were removed under ether anaesthesia. The uterine horn had increased in size, being 12 mm. in mean diameter, while Gartner's duct had become smaller (2 mm. in

diameter). The wounds, which as before were closed by means of catgut, healed rapidly.

A part of the right testis was fixed in Helly's and in Zenker's fluid, the rest of the specimens being placed in Bouin's fluid.

Period of castration. During the 4 months following the third operation, apparent symptoms of heat were noted twice (27 November and 17 December 1937).

The animal was killed on 3 March 1938. It was then 19½ months old and weighed 480 lb.

HISTOLOGICAL OBSERVATIONS

Control observations

The object of the investigation was to examine the gonads and internal reproductive organs under different conditions of endocrine stimulation, and since pieces of the uterus had to be taken from different regions, it was first necessary to enquire whether or not the histological appearance of the cornua is uniform throughout their length. Three normal control sows were therefore examined, five segments of the cornua from arbitrarily chosen sites between the Fallopian tubes and the corpus uteri being prepared histologically. The ovaries of the first animal contained fully mature corpora lutea, those of the second contained younger corpora lutea, while those of the third contained very recently ruptured follicles (pieces of each ovary were examined histologically).

Measurement showed that from about 5 to 10 cm. from the junction of the cornua with the Fallopian tubes, the diameter of the cornua remained fairly constant as far as the corpus uteri. It was also found that the appearance of any one part of the uterine horn is characteristic of that of the entire horn, with the one main exception that the degree of plication of the superficial and relatively non-glandular zone of the endometrium becomes less as the junction of the horn and Fallopian tube is approached. In general, the appearance of the three specimens examined corresponded with the descriptions of the pig uterus given by Corner (1921) and McKenzie (1926). They appeared to differ in the following two respects only: (1) the endometrium was markedly oedematous in spite of the fact that corpora lutea were present in the ovaries, (2) the superficial part of the endometrium was arranged as a lace-work of numerous prominent crypts. This lace-work appearance was very pronounced in the animal whose ovaries contained the recently ruptured follicles.

EXPERIMENTAL OBSERVATIONS

(1) *Uterus*

(i) *The intersexual uterus at the start of the experiment.*

The uterine lumen is irregularly stellate as a result of the projection into it of endometrial cushions of unequal size, and its main axis runs from the mesometrial to the antimesometrial borders (Fig. 1). The mucosa is very

oedematous, and its numerous tubular glands are mostly concentrated in its deeper zone. The lumen contains eosinophilic secretion in which no cellular elements were observed. The cross-sectional diameters of the cornu are almost twice as small as those of any of our control specimens.

The surface epithelium is composed of moderately tall columnar cells with their nuclei either in the middle or basal thirds of the cell bodies (Fig. 10). As in the normal oestrous sow (Corner, 1921), the arrangement of the cells is occasionally "deceptively suggestive of stratification", an appearance which probably owes its origin to oblique cuts across the irregularly disposed crypts. Mitoses are very frequent.

Almost all the tubular glands have a clearly defined lumen which, in a few cases, is distended with secretion (Fig. 6). The glandular epithelium is in general lower than that of the cavum uteri, and most cells have basally disposed nuclei. In a few cases the nuclei are alternately in the middle and basal thirds of the cells, and such glands have the appearance of being lined by two layers of cells. There are relatively fewer mitoses in the epithelium of the glands than in that of the cavum uteri. Some glands are present in the myometrium, where a few were also seen in our control specimens.

The oedematous stroma is relatively avascular, and occasional leucocytes, and in particular eosinophils, lie free within it.

(ii) *The intersexual uterus after androgenic stimulation.*

The segment of uterus removed after the period of male-hormone treatment was, as noted above, 12 cm. from the termination of the right cornu on the testis. Our control observations show that, at this distance from the Fallopian tube, the mean diameter of the uterine horn is normally not much less than that of more medial parts. The relatively small diameter of the piece of uterus removed after androgenic stimulation indicates, therefore, that there was a considerable reduction of the size of the whole uterine horn. The cross-sectional area after androgenic stimulation (measured from projections of the sections) was slightly less than half that of the segment removed previous to experimental treatment. The cross-sectional area of the segment removed after oestrogenic stimulation (see below), which was taken from the opposite uterine horn in the region corresponding to that from which the specimen was removed after androgenic treatment, was also slightly more than twice that of the specimen representative of androgenic stimulation.

The uterine lumen is relatively more open than in the previous specimen owing to the reduction in size of both the endometrial cushions and the intervening crypts (Fig. 3). The endometrium is much more shallow and less oedematous than in the previous specimen. It is also relatively less glandular, and tubular glands have disappeared almost completely from the mesometrial zone of the endometrium. Moreover, there is no longer any clear differentiation of a deeper glandular and a more superficial non-glandular zone, for in the regions in which they occur, tubular glands approach almost to the epithelium

of the cavum uteri. That this change was a definite effect of the androgenic treatment is suggested by the fact that the corresponding segment of uterine horn removed from the opposite side after oestrogenic treatment was similar in structure to the specimen removed before experimental treatment.

The cavum uteri contains a coagulum of eosinophilic secretion in which are to be seen numerous leucocytes and shed epithelial cells. The surface epithelium is typically composed of a single layer of tightly packed columnar cells somewhat smaller than those of the previous specimen, and with nuclei which usually fill the basal halves of the cells (Fig. 12). In a few places the nuclei are superficial in disposition, and the deeper parts of the cell have a clear cytoplasm similar to that which is observed in the primate uterus when glycogen is deposited in the cells as a result of oestrogenic stimulation. No breaches were observed in the epithelium, nor were there any mitoses. The eosinophilic material in the cavum uteri was presumably the product of secretion of the surface cells, of the crypts and of the tubular glands. In several places leucocytes could be seen in progress of migration through the epithelium.

The endometrial glands were in general much more embryonic in character than in the previous specimen (Fig. 8). Some of them showed a clear lumen. Those that did were often lined by two to three layers of small cells. A few still retained their normal lining of columnar epithelium, and in some places distended glands were encountered with usually a more flattened epithelium. Other glands appeared as cords of epithelium with obliquely directed lateral buds. A few mitotic figures were observed in the gland system.

The stromal cells are much more tightly packed together than in any of the other sections of pig uterus examined. Within the stroma can also be recognized wandering white blood cells, many of which are eosinophils. The uterus is moderately vascular.

No tubular glands were observed within the myometrium.

(iii) *The intersexual uterus after oestrogenic stimulation.*

As noted above, the segment of the uterus removed after oestrogenic stimulation was taken from the left side, and from a region corresponding to that from which the specimen was removed after androgenic treatment. Its cross-sectional diameter was slightly more than twice that of the latter specimen, and almost equal to that of the segment removed at the start of the whole experiment.

With the following exceptions the appearance of the uterus is identical with that of the first specimen which was removed (Fig. 2).

(1) The surface epithelium is a more regularly disposed single layer of columnar cells, somewhat smaller in size than in the control (Fig. 11). The nuclei occupy a very large part of the cell body in spite of the fact that the cells appear to be actively secreting. The nuclei are also more uniformly disposed, and only the free margins of the cells show clear cytoplasm. Many fewer mitoses were observed.

(2) The glands are tubular but less uniform in character than those of the control. Some have practically no lumen, while others are distended (Fig. 7). The disposition of the epithelium also varies. In some it is a regular low columnar epithelium, in others it is flattened, while in others again it is similar to that of the more embryonic glands observed in the specimen removed after androgenic stimulation. Only a few mitotic figures were encountered in the glandular epithelium.

(3) In places the oedema is so marked that an entire field observed under a $\frac{1}{6}$ in. objective is free from fixed stromal elements. As in the control specimen, the oedema is most marked in the superficial zone of the endometrium (in which the endometrial cushions are developed).

(iv) *Intersexual uterus 4 months after removal of the gonads (specimens obtained after autopsy).*

The segment of uterus examined histologically after removal of the gonads was taken from the region corresponding to that from which the original control specimen was removed. Its cross-sectional area was only 70 % that of the original specimen.

The uterus shows all the signs of widespread involution. The endometrial cushions are reduced, although the uterine lumen is still irregularly stellate, with its long axis running from the mesometrial to the antimesometrial borders (Fig. 4). The endometrium is not oedematous. It is deeper than in the specimen removed after androgenic stimulation, and, unlike the latter, the individual glands, although much more involuted and embryonic in character, are fairly uniformly disposed on all sides of the lumen. The demarcation of endometrium and myometrium is more indistinct and irregular than usual, but, as is usual, the most superficial part of the endometrium is relatively free of glands.

The epithelium of the cavum uteri, which does not appear to have contained much secretion, consists of regularly disposed, moderately low columnar cells whose nuclei fill most of the cell body (Fig. 13). The basal cytoplasm of the cells is often vacuolated. There are no breaches in the epithelium, and very few mitotic figures were encountered. Many leucocytes, however, are to be seen migrating through the epithelium. The lumina of the glands are very much reduced and, in the deeper parts of the endometrium, completely obliterated. In the latter cases the glands are represented merely by cross or longitudinal sections of tracts of almost undifferentiated and irregular epithelial cells (Fig. 9). In the more superficial zone the glandular epithelium still retains its columnar character. The nuclei of the cells, which even in these regions are irregularly disposed, occupy the greater part of the cell bodies.

The stroma, which is relatively more avascular, is very fibrous, and its cellular concentration is greatest in the most superficial zone, the zone which normally is differentiated into very oedematous endometrial cushions. It is most fibrous in the middle and deepest third.

A similar picture of involution in the uterus of castrated intersexual pigs has been observed by Krediet (1930*a*).

(2) *The vas deferens*

(i) *The intersexual vas deferens at the start of the experiment.*

The vas deferens has a relatively small lumen, and the muscular coat makes up the greater part (92·4 %) of the total cross-sectional area of the duct (estimated by measurements of projections of the sections). The mucosa consists of a much folded layer of tall columnar cells set upon a basal layer of small cuboidal cells with relatively very large nuclei (Fig. 14). The folding of the layer of columnar cells, which have no obvious ciliae and whose oval nuclei are in general centrally disposed, gives the lumen of the duct a stellate appearance. There is very little secretion and the sub-mucosal region is oedematous.

(ii) *The intersexual vas deferens after androgenic stimulation.*

The cross-sectional area of the vas, measured from projections of sections, is five and a half times as great as that of the specimen removed at the start of the experiment, and the muscular coat now makes up only 74·5 % of the total area. Reduction of the muscle area/total area ratio is also a characteristic effect of androgenic stimulation in the seminal vesicles of monkeys (Zuckerman & Sandys, 1939).

The lumen is widely open, elliptical in shape, and filled with an eosinophilic secretion which contains practically no cellular elements. Folds in the lining epithelium are still present, but they are insufficiently marked to do more than give a slightly wavy outline to the lumen. The lining epithelium consists of very tightly packed, actively secreting columnar cells, which appear to be taller on the summits of the folds than in the depressions between them (Fig. 16). Their oval nuclei are small in relation to the cytoplasm, and are usually irregularly disposed either in the basal or middle zones of the cells. Occasionally they lie more superficially. As in the control specimen, a layer of small cells appears to be present deep to the cylindrical epithelium. The submucosa, on the other hand, is much less oedematous, and both it and the muscular coat are relatively more vascular.

The general appearance of this specimen is identical with that of the vas of a normal mature boar which was studied for purposes of comparison.

(iii) *The intersexual vas deferens after oestrogenic stimulation.*

As in the case of the uterus, the segment of vas removed after the period of oestrogenic treatment was taken from the left side, and from the region corresponding to that from which a piece was taken (from the opposite side), after the phase of androgenic treatment. The total cross-sectional area of the specimen is one-fourth that of the latter, though 1·4 times as big as the piece removed at the start of the whole experiment. Presumably the effects of the

previous androgenic treatment had not completely worn off. On the other hand, the muscle area/total area ratio had returned to 90·0 %.

The general appearance of the specimen is identical with that of the piece removed at the start of the experiment, with the single exception that the submucosa appears to be more vascular (Fig. 15).

(iv) *The intersexual vas deferens 3½ months after removal of the gonads.*

As in the case of the uterus, the specimen of vas removed after the animal was castrated was taken at autopsy from the region immediately adjacent to that which provided the segment examined at the start of the whole experiment. Involution of the vas appears to have taken place, for its cross-sectional area is only 77 % that of the original control specimen. Its muscle area/total area ratio is, however, the same. This finding suggests that the process of atrophy has affected the muscular and epithelial coats equally.

The epithelial folds are intermediate in prominence between those of the first and third specimens and those of the segment removed after androgenic treatment, while the lumen is, in general, elliptical in appearance, with irregular wide crypts. It contains very little secretion (Fig. 17).

The lining epithelium is much lower than in any of the previous specimens, and the nuclei of the low cylindrical cells fill most of the cell bodies. A basal zone of small cells can still be distinguished.

The submucosa and muscularis are very avascular, but the former is much more oedematous than it is in the specimen removed after the phase of androgenic treatment.

Testis

The gonads require examination at three phases of the experiment—at its start, at the end of androgenic stimulation and at the end of oestrogenic treatment, when the animal was gonadectomized. The specimens which were prepared show remarkably few differences. As stated above, no trace of ovarian tissue was found in any of the numerous sections which were cut. The gonads consist entirely of swollen polyhedral interstitial cells and of seminiferous tubules lined by a single layer of large vacuolated cells, and apparently distended with a vacuolated but otherwise homogeneous non-staining substance (Fig. 24). There was no evidence of any phase of spermatogenesis. Moreover the condition of the seminiferous tubules does not appear to have varied in any of the phases of the experiment, nor has any obvious change occurred in either the number or character of the interstitial cells. In every respect, and during all phases of the experiment, the latter appear to have been identical with the normal interstitial cells of intersexual pigs, as described by Baker (1925*b*). They show a remarkable resemblance to luteal cells, and to the cells of the adrenal cortex. Their ovoid or spherical nuclei are mostly eccentric, being pushed to one side by a central deposition of deeply staining granules. The differentiation of the cytoplasm into a peripheral

clear zone and a central eosinophilic region is obvious even in haematoxylin-eosin preparations. It is however more marked in cytological preparations stained by Volkonsky's method for mitochondria. The central lipoid granules vary in quantity from cell to cell. In some cells they are either sparse or absent, but they can be made out in most, and in a few they appear to fill the entire cell bodies. No mitoses were observed either within the interstitial elements or the seminiferous tubules in any of the specimens. Columns of interstitial cells and occasional seminiferous tubules were observed within the tunica albuginea. Testicular tissue outside the tunica of the degenerate testes of intersexual pigs has been described by Crew (1924).

Although no changes occurred in the appearance of the interstitial cells, measurement shows that changes actually did take place both in their size and in their nuclear-cytoplasmic ratios. In each specimen twenty cells were taken at random, and drawn, with the help of a camera lucida, under an oil immersion lens. Nuclear and cell areas were measured with a planimeter. It was found that the size of the cells was not significantly larger, as estimated by statistical methods, at the end of the phase of androgenic stimulation than at the start of the experiment, but that the nuclear-cytoplasmic ratio was almost significantly greater ($n=20$; $t=2{\cdot}364$; $P=0{\cdot}02$–$0{\cdot}01$). On the other hand the cells had significantly increased in size after oestrogenic treatment (P in both cases being $<0{\cdot}01$), while the nuclear-cytoplasmic ratio had decreased significantly. This observation suggests that the interstitial cells were either elaborating or storing more of their secretion during the period of oestrogenic treatment. Burrows (1936) and Gardner (1937) have both reported an increase in the size of the interstitial cells of male mice which had been injected with oestrogenic substances, and both observers also believe that there is an increase in the number of the cells.

It may be noted that Krediet (1933), who has made a close study of the cytology of the gonads of intersexual pigs, regards the single layer of vacuolated cells in seminiferous tubules as "indifferent" epithelium and not as cells of Sertoli. Following Regaud (1910) he also regards the cells as syncytial in character. On the other hand most workers (e.g. Hanes, 1911; Moore, 1939) regard these cells as normal cells of Sertoli.

OTHER REPRODUCTIVE ORGANS

(1) *Female organs*

(*a*) *The corpus uteri 4 months after castration.*

The stroma of the corpus uteri is dense, and the surface epithelium and the epithelium of the crypts are composed of a single layer of columnar cells of medium height with oval nuclei which fill the basal halves of the cells. There are few tubular glands within the stroma. The lumen of the corpus uteri is filled by a large multilobulated non-glandular polypoid growth (Fig. 20).

(*b*) *The vagina 4 months after castration.*

The vagina is lined by an epithelium of somewhat irregularly disposed cuboidal or columnar cells (Fig. 5) which, especially in the lower part of the organ, often overlie one or two layers of small, irregular, deeply staining epithelial cells. The mucosa is folded, and, between the major crypts formed by the folding, glandular downgrowths of the deeper staining basal cells occur. Some of these glandular growths are obviously involuted and are not fully canalized. Others are so well-developed that secondary tubular glands lined by cuboidal or cylindrical epithelium have budded from them (Fig. 23). Most of the deep crypts in the lower part of the vagina are lined, like the surface epithelium, by a superficial layer of columnar cells and a deeply staining basal zone from which secondary tubular glands spring.

The submucosa is fibrous and oedematous, and the surface of the mucosa is often raised by fibro-muscular, non-glandular, excrescences similar in structure to that of the polypoid growth of the corpus uteri.

This glandular appearance of the vaginal mucosa is altogether dissimilar from the vaginal epithelium of normal sows (as described, for example, by McKenzie (1926) and also in our own specimens). It possibly represents a reversion to an undifferentiated state, for, with the exception of the glandular crypts and tubular glands, it appears to be identical with that of the intersexual shrew described by Brambell & Hall (1936). An alternative interpretation is that the glandular vagina, which, as noted above, was not clearly demarcated from the corpus uteri, represents a primitive Müllerian epithelium unaltered by admixture with epithelium of the urogenital sinus.

(2) *Male organs*

(*a*) *Epididymis at the end of the period of oestrogenic stimulation.*

The tubules of the epididymis are lined by very tall, ciliated, tightly packed cells with basal, oval nuclei (Fig. 19). They are much more distended and filled with secretion in the tail than elsewhere, and in this region the lining cells are not as tall as those in the head or body of the organ; in some of the more distended tubules they are cuboidal. The thickness of the muscular coat in the head and body is less than the height of the epithelial lining, but a reverse relationship holds in the tail.

(*b*) *Vesicular gland 4 months after castration.*

As noted above, the vesicular glands in our experimental animal were actually ampullary glands of the vasa deferentia.

In their general anatomical relationships and in histological structure they resemble, however, the seminal vesicles of normal boars.

The lobules of which they consist have a thick muscular coat, and the main lumina are very irregular in shape, numerous crypts leading from them. The lining epithelium consists of a regularly disposed layer of medium-sized

columnar cells whose oval nuclei fill the greater part of the cell bodies (Fig. 25). The lumina contain a moderate amount of eosinophilic secretion in which are to be seen shed epithelial cells and leucocytes.

The main differences between the histological appearance of the vesicular gland in our experimental animal and that of the seminal vesicle of a normal boar are the relative absence of secretion, the lower height of the epithelium, and the relatively greater amount of muscular tissue. In these respects our specimen resembles the seminal vesicle of a castrated boar.

(*c*) *Prostate* 4 *months after castration.*

It has already been noted that an obvious prostate was present in the angle between the urethra and the vagina, and that prostatic glands are also to be recognized within the wall of the upper part of the urogenital sinus.

The prostatic acini are identical in histological structure in the two sites, and resemble those of the prostate of a castrated boar. Each prostatic lobule has a central duct that is lined by low columnar or cuboidal epithelium, and which is surrounded by closely packed acini lined by cuboidal cells (Fig. 18). Many of the acini do not appear to have a central lumen, and their lining cells have a clear cytoplasm.

(*d*) *Urogenital sinus* 4 *months after castration.*

As already noted, the lining epithelium of the urogenital sinus after castration consists mostly of low stratified epithelium (Fig. 22).

DISCUSSION

The pig that was the subject of the present enquiry was a sex-intergrade which possessed, as is shown by the anatomical observations reported above, (*a*) gonads of purely testicular character, with no evidence of previous or present ovarian tissue, and (*b*) full complements of the accessory reproductive organs of both sexes. Our experimental and histological observations make it clear, as the following brief analysis indicates, that during life the animal's gonads were producing only oestrogenic hormone, or if androgenic hormone as well, in such low amounts that its effects were completely masked.

(*a*) In some circumstances androgenic stimulation can cause growth and differentiation of the uterus (rats, rabbits, e.g. Korenchevsky & Hall, 1937; Phelps *et al.* 1938; Courrier & Gros, 1938). In the present experiments the picture of the uterus after injections of testosterone propionate was one of involution, similar in character to that observed in the rhesus monkey after the same treatment (Zuckerman, 1937). It was completely different from the histological picture of the uterus at the start of the experiment, and that, on the other hand, was practically identical with the appearance of the endometrium after oestrogenic treatment. It follows therefore that, before the experiment was started, the animal's uterus was responding to a predominantly oestrogenic stimulus. Presumably this stimulus was not as intense as it is in

normal sows, since the intersexual's uterus was smaller than normal. Other possible explanations for the latter fact are (1) immaturity, and (2) some peculiarity of the fibromuscular and epithelial tissues of the intersexual uterus, imposed upon them by their abnormal differentiation, which prevents them from responding fully to a normal oestrogenic growth stimulus.

(*b*) The condition of the vas at the start of the experiment was similar to that at the end of oestrogenic stimulation, and altogether different from the histological picture presented after treatment with androgenic hormone. It was also different from the involuted appearance observed after castration. Oestrogenic hormone stimulates the growth of fibro-muscular tissue in most of the male accessory organs, and the difference in the vas between the ratio of muscle cross-sectional area to total cross-sectional area after androgenic and oestrogenic stimulation respectively is of the same kind as is observed in the seminal vesicles (e.g. Freud, 1933; Zuckerman & Sandys, 1939). These considerations indicate clearly that before experimental treatment was begun the vasa deferentia of the intersexual were under the influence of oestrogenic, and not androgenic, stimulation. It is idle to speculate about the chemical nature of the oestrogenic hormone concerned. Even if it was an ambisexual "male hormone", it is perfectly plain that its androgenic was much less intense than its oestrogenic potency. In the circumstances, the active appearance of the epithelium of the epididymis after oestrogenic stimulation is rather curious—unless it be that the cells have an even more active appearance after androgenic stimulation.

It is also perfectly clear that during the period of androgenic treatment the gonads were either not elaborating any oestrogenic hormone at all, or that what oestrogenic hormone was produced was immediately neutralized by the injected male hormone. On the basis of currently accepted views about the inhibitory effect which androgenic treatment has upon the gonad-stimulating capacity of the anterior lobe of the pituitary (see Fevold, 1939), the former is the more likely explanation, and one which harmonises with the fact that the amount of cytoplasm in the cells was almost significantly reduced (P = between 0·02 and 0·01). On the other hand, the significant increase in the size of the cells, and the significant decrease in the nuclear-cytoplasmic ratio, under the influence of oestrogenic stimulation, suggests, as already noted, either an increase in the rate of secretion of these cells, or at least an increased storage of secretion. This finding is in keeping with another widely accepted hypothesis that oestrogenic hormone stimulates the anterior lobe of the pituitary to produce its luteinizing hormone, for the latter, as many studies have shown, activates the interstitial elements of the testis.

One further point requires mention before the more general implications of our findings are considered. After the gonads were removed both the male and female accessory organs involuted. This fact indicates that, apart from the gonads, the intersexual pig possessed no effective source (e.g. adrenal cortex) of either androgenic or oestrogenic hormone.

It is difficult to align these observations with current theories about the origin of intersexuality. The more remote explanation of these conditions in domestic pigs deals with their hereditary background (e.g. Crew, 1923, 1926; Baker, 1925*a*). In a recent review of the subject, Witschi (1939) states, however, that the data which concern this aspect of the problem "are entirely unsatisfactory". Baker's view (1925*a*) is that the boar is responsible for the genic disturbance which underlies the abnormality. The only observation in favour of this hypothesis that is regarded by Witschi as being significant is Krediet's record (1930*b*) of the son of an intersex-producing boar which also sired intersexes with both related and unrelated sows. The family history of our specimen is a somewhat similar case (see p. 173). It does not, however, add sufficiently to the genetical data to make the latter worthy of any more consideration than is afforded them by Witschi. On the other hand, our experimental and histological observations add considerably to the data which are available for evaluating the morphogenetic basis of the condition.

The accepted view (Willier, 1939; Witschi, 1939) about the normal morphogenesis of the gonads and accessory reproductive organs in mammals can be briefly stated as follows. The gonad rudiments are specifically organized as to sex in accordance with the genic constitution of the zygote from which they derive. The fact that they are bipotential sexually is normally of no consequence, for the hormonic stimulus which leads to their ultimate differentiation is also in accordance with the sexual genic constitution of the zygote. The soma, on the other hand, is manifestly neutral or bipotential, and anlagen of both male and female reproductive systems, the Wolffian and Müllerian ducts, and of the ambisexual urogenital sinus, develop in both sexes. Their earlier differentiation is not under endocrine control (period of "self differentiation" or "sexually indifferent" period), but their subsequent development depends upon the direction taken by the gonads. If these differentiate into testes, the secretions of the latter stimulate the further development of the Wolffian system and suppress that of the Müllerian, which usually disappears except for vestiges at its cranial (hydatids of Morgagni) and caudal (uterus masculinus) extremities. If they differentiate into ovaries, the Wolffian system is inhibited (as a rule disappearing entirely), and the Müllerian apparatus is stimulated to further differentiation and development by the ovarian secretions.[1] The fate of the urogenital sinus also depends on the course taken by the gonads in their development.

Aberrations of this normal morphogenetic process can best be explained as being due to aberrations in the process of endocrine stimulation. For example, when the foetal membranes of twin male and female cattle embryos become united so that the two foetal circulations anastomose, the differentia-

[1] Wiesner (1935) has put forward a "monhormonic" theory of sexual differentiation, according to which the female differentiates in virtue of her genic constitution alone. This view is based upon the supposition (which is not supported by recent work) that oestrogenic hormone has no morphogenetic action.

tion of the gonads of the female twin is influenced by the secretions of those of the male twin (which are the first to become differentiated and functional), and their further development is such that they either remain "rudimentary gonads" without characteristic male or female features, or they become typical testes with the one exception that the seminiferous tubules do not function. As a result of the androgenic secretion of the transformed gonads, the Müllerian apparatus is suppressed and the Wolffian develops—"the degree of masculinization of secondary sex characters" being, according to Witschi and Willier, "directly correlated with the more or less testicular character of the free-martin gonad".

It is against this background of well-established fact and theory that the present discussion has to be ranged. The first important point which arises is that the testes of the experimentally studied sex-intergrade were apparently producing, not androgenic hormone, but only, or mainly, oestrogenic hormone. If an organ which is histologically testicular in character can behave in an ovarian way during post-natal life, there is no reason for supposing that it would be incapable of doing so during foetal life. Consequently, it is unnecessary to postulate as Baker (1925*a*) did (in order to account for the stimulus which is presumably responsible for the differentiation and development of the accessory female reproductive organs) that at one time the foetal (or infantile) gonad was partly and definitively ovarian in character.

Baker's view is that all sex-intergrade pigs are masculinized genetic females which at some time developed both ovarian and testicular tissue, and unlike Crew (1924) he regards intersexuals that possess both ovarian and testicular tissue as being no different in kind from those whose gonads are composed of testicular tissue only. On this hypothesis the two types represent different phases of the same process in the transformation of genetic females. Crew's alternative opinion is that intergrades of the kind described in the present paper are genetic males whose gonads began to function as endocrine organs later than normal, and that intergrades which possess both male and female gonadal tissue are genetic males of an entirely different class whose gonads, when differentiating, responded to both "quickly elaborating female-determining factors and slowly elaborating male-determining factors". On this view one of the classes of intergrade is "the result of an abnormality in the time of differentiation of the gonads, and the other of abnormality in the mode and in the time of differentiation". In a later publication, however, Crew (1926) leans to the view that the second class of intergrade is actually, as Baker maintained, genetically female. This view clearly makes an even greater distinction between the two classes. Krediet (1930*a*, *b*) also holds that most male intersexuals are genetic females.

Only negative evidence forms the basis for the belief that the first type of intergrade is genetically male. As Crew writes, there is "no evidence that such animals represent free-martins, cases of sex-reversal in a genetically determined female, or cases of true glandular hermaphroditism in which the

ovarian tissue had been removed at an earlier stage of development". The evidence in favour of the view that they are genetic females is, however, even less substantial, and, as noted above, depends upon the occasional presence of ovarian tissue in the gonads of pig sex-intergrades.

Although he appears to endorse Baker's views on pig sex-intergrades, Witschi (1939) has advanced a theory with regard to human intersexuality which seems equally capable of fitting the facts about intersexuality in pigs, and which has the advantage of taking into account more recent developments in our knowledge of sexual differentiation. This theory depends on the established finding that the placenta is sometimes permeable to female sex-hormone derived from the maternal organism (for references see Zuckerman & van Wagenen, 1935). Witschi suggests that human male intersexuality is primarily due to the genetical constitution of the mother, and that the abnormality results from the passage of an excess of "maternal hormone" to the developing embryo. Thus in crucial cases of intersexuality "which combine fully or partially sterile testes and male gonoducts with a blind vagina" together with external feminine characteristics, the first part of foetal development was the normal male type. "Later there occurred feminization causing the last developed secondary sex characters to assume a prevailingly female type." In addition to this type of "crucial" intersexual, there is another in which a phase of secondary masculinization follows that of feminization.

If these views on male intersexuality in Man are extended to pigs, it would follow that sex-intergrade pigs are feminized genetic males. Witschi's theory, however, requires qualification. For example, elsewhere in his comprehensive review he makes the point that the testes of human pseudohermaphrodites produce "considerable amounts" of oestrogenic hormone, and this fact he regards as indicating that the sex-hormone system of the developing male foetus is "reversed" by the mother. "After birth", he writes, "this reversal is only partly, and in individually varying degrees, restored to normal." This view is clearly too rigid, for our observations indicate that there may be substantially no reversal, and that organs which are manifestly testes may produce only (or predominantly) oestrogenic hormone.

It is therefore suggested here that the most economic of hypotheses which can be advanced to explain those cases of intersexuality that appear to be primarily dependent upon gonadal changes (i.e. types in which the adrenal cortex, for example, is not involved, see Young, 1937) is that both types of sex-intergrade defined by Baker & Crew are feminized genetic males. In these animals the normal course of gonadal development has been interfered with as a result of some abnormality which allows a significant amount of maternal (female) sex-hormone to pass into the foetus. This change occurs during a very brief critical interval either before or after the disappearance of the cortical germinal zone from which, if the gonad were genically female, cortical or Pflügerian cords differentiate. Usually it seems to take place after the disappearance of this zone, and as a result the gonads develop into degenerate

testes, which may become predominantly oestrogen-producing organs, and which, apart from interstitial cells, contain only degenerate seminiferous tubules. More rarely the change occurs earlier, and the cortical zone differentiates into ovarian tissue that becomes separated by the primitive (and definitive) tunica albuginea from the main medullary mass of the gonad, which has already differentiated into a testicular organ. When this happens an ovotestis results.

That the differentiation of the embryonic mammalian gonad can be altered is shown by the development of the free-martin, although in this case a genically female gonad is changed in a male direction. The rarity of the development of ovarian tissue in the gonads of intergrade pigs, as compared with the constancy of the male gonadal change in true free-martins, is in keeping with the well-known observation that sex-reversals from female gonad to male gonad are much commoner in experimentally induced intersexuality (especially in amphibia) than is the reverse change.

This view is clearly more economic than the contrary one that in pig sex intergrades a fundamentally ovarian gonad is usually transformed entirely, and only occasionally incompletely, into testicular tissue. Moreover it suggests that a main difference between the development of cattle free-martins and the development of pig male sex intergrades lies in the source of the hormone which alters the chemical environment of the developing gonad. In the case of the differentiating gonads of the female free-martin twin the chemical disturbance originates in the androgenic secretions of her male twin. In the case of male intergrades the chemical disturbance is the abnormal passage of maternal female sex-hormone into the foetal circulation.

It is necessary to test the applicability of this hypothesis to pig sex-intergrade gonads of the kind described by Brambell (1929, 1930). These gonads, which in general were testicular in character, possessed a cortical zone that on one side was purely testicular and on the other a combination of ovarian and testicular tissue. Indeed the intermixture in this region was so marked that recognizable oocytes could be made out within the degenerate seminiferous tubules. Brambell's interpretation of gonadal development is that the stimulus to effective male differentiation of the gonad becomes operative "as soon as the medullary cords and the primitive tunica are formed, and before the cortical proliferation is due to commence. If this stimulus was somewhat delayed we might suppose that the cortical cells would have begun to form, outside the primitive tunica, before it was effective.... Once formed their further development into ovarian or spermatic tissue would depend, probably, on the time of appearance of the male-determining stimulus. If this stimulus were effected at once the cortical cords would be transformed into a zone of spermatic tubules outside the primary tunica albuginea.... If the stimulus was delayed still further a greater or lesser part of the cortex would differentiate into ovary."

This view does not conflict with the hypothesis which we have advanced,

but it has the limitation that it fails to take into account the fact, discovered since the formulation of the problem by Brambell, that the differentiation of the cortical and medullary parts of the gonad is determined by androgenic and gynaecogenic "inductors" which are hormonal in nature (Witschi, 1939). Since this fact must be considered, it becomes necessary to suppose, in order to explain the gonads described by Brambell, that the stimulus to cortical differentiation was followed by a transient phase in which androgenic stimulation, from the medullary part of the gonad, almost mastered the course of development of the cortical cap of ovarian tissue. This transient phase was then succeeded by a period in which the differentiation of the gonads once again came under the influence of maternal sex-hormone.

It should be noted that the hypothesis developed here is opposed to Dantchakoff's much-reiterated belief (1937, 1938, 1939) that oestrogenic hormone can have no effect on sexual morphogenesis in the male mammal because of its supposedly toxic and lethal influence on the embryo. This view, while supported by some earlier studies, is in complete disagreement with Raynaud's (1939) and Greene *et al.* (1938*a*) recent and conclusive findings that oestrogenic hormone in fact has a very profound morphogenetic effect, even though, so far as is known at present, it does not lead to transformation of the gonads. If Dantchakoff's theories were applied to the interpretation of the animal which formed the subject of our study, it would have to be supposed that the creature was a masculinized genetic female. Yet neither she, nor Raynaud in his earlier studies with male hormone, has succeeded (with the exception of one set of observations) in causing a greater change in the female gonad than the development of a medullary rete. Indeed, Dantchakoff herself (1939*b*) has been able to show, by means of artificial insemination, that the gonads of experimentally masculinized female guinea-pigs produce fertile ova, in the same way as the gonads of feminized cocks produce fertile sperm (1939*a*). The only contrary observations which Dantchakoff has reported (1939*c*) concern the gonads of three genetic female embryos in whom medullary development, as a result of the injection of male hormone on the 13th day of gestation, was more extensive than is usual after such treatment. Apart from a rete testis, these gonads possessed a central testicular zone in which were cords of cells containing occasional large round cells which Dantchakoff refers to as "des cellules mères des spermatogonies futures". This central medullary zone was surrounded by a cap of ovarian tissue.

These are the only experimental observations which suggest that a genetically female mammalian gonad could be transformed into a testis, and the degree of change which they imply is far removed from that which would have to be accounted for if the gonads of our experimental animal were those of a genetic female. But in any event, if they were those of a genetic female, the insoluble problem presents itself of discovering a source for the androgenic stimulation which would have been necessary to transform the gonads. The abnormal placental conditions necessary for the formation of a free-martin are extremely

rare in pigs (Hughes, 1927, 1929), and most cases of "hermaphroditism" in this species are certainly not of this kind. Furthermore, if the animal were a true free-martin, the extent of testicular change hardly suggests that the Müllerian system would have survived during the period of transformation to the degree it did, for in cattle free-martins the system is no better developed than it is in normal males. It is of interest in this connexion, however, that both Dantchakoff and Raynaud's observations on masculinized females show that, like normal ovaries, the ovaries of such animals secrete oestrogenic hormone. This fact is clearly demonstrated not only by the occurrence of typical oestrogenic changes in the uterus and vagina, but also by metaplastic effects in such organs as the coagulating glands.

The difficulties which the hypothesis advanced here has to face with regard to the differentiation of the accessory reproductive organs appear to be less than those which are met with in other theories of abnormal gonadal differentiation. All theories have of course to assume that the alteration in the direction of differentiation occurs, during a very critical interval, before the Müllerian and Wolffian derivatives, and the urogenital sinus and external genitalia, have undergone the definitive changes which they normally do. Crew's exposition of the problem states the issues very clearly, even though his hypothesis requires some modification in the light of recent knowledge. According to him if "proper endocrine control" is lacking during the period of differentiation of the external and internal reproductive organs, both the Müllerian and Wolffian ducts undergo undirected growth and develop into the various organs to which they normally give rise. The absence of the "proper endocrine control" he regarded as due to "the qualitative or quantitative insufficiency of the tissue in which the sex-hormone has its origin", and in the case of animals such as that which formed the subject of the present study "it was assumed that the differentiation of the embryonic gonads into testes occurred later than usual during development, during or after, instead of before, the time when differentiation of the rest of the sex-equipment occurs". If the gonads start functioning as testes after the Müllerian duct has undergone undirected differentiation and lost its embryonic plasticity, the Müllerian organs will persist side by side with an activated male reproductive tract. This view assumes that three overlapping phases normally occur in the differentiation of the male reproductive organs: (1) the atrophy of the Müllerian ducts; (2) the further development of the Wolffian duct derivatives; and (3) the modelling of the external genitalia.

Shortcomings in this hypothesis are indicated, by implication, in the alternative explanation which is offered below. But at this point two very important general issues which the hypothesis does not take into account may be mentioned. First, it assumes that the uterus in sex-intergrade pigs of the kind studied by us is at no time acted upon by the gonadal secretions, which, as we have shown, is contrary to the facts. And second, the hypothesis does not make allowance for the possibility that the developing foetus may in

some cases be exposed to sufficient amounts of maternal oestrogenic hormone to alter significantly the chemical environment within which it is differentiating.

The view presented here is that the Wolffian ducts, in sex-intergrades of the kind we have studied, had differentiated (presumably under the influence of some kind of androgenic stimulation from the gonads) before the Müllerian apparatus had atrophied, and before the urogenital sinus and external genitalia had been moulded in a masculine direction. Before this critical phase had passed, the character of the gonads had begun to change as a result of stimulation by sex hormone originating in the maternal organism, and the hormone which the transformed gonads then elaborated, as well as maternal hormone, acted upon the Müllerian ducts, urogenital sinus, and external genitalia, which consequently developed in the direction normal to females. According to this hypothesis the gradation of persistence of Müllerian structures, and the degree of masculinization of the external genitalia, are in the first place an index of the interval which intervenes between the beginning of normal differentiation of the gonads and the start of the abnormal change in their development. If this interval is long, more of the Müllerian apparatus would have disappeared, and the external genitalia would have been further moulded in a masculine direction. The variation in the degree of development of Müllerian characters is in the second place a measure of the intensity of the feminizing stimulus originating from the maternal organism and the transformed foetal gonads. It is, of course, unnecessary to assume that the intensity of the maternal stimulus and the switch in the hormonal character of the gonad are always as complete as they appear to have been in the intergrade which we studied.

This hypothesis has the merit that it takes into account the fact that, on the one hand, sex-intergrade reproductive organs are undoubtedly influenced by oestrogenic hormone, and that on the other they need not show any evidence of stimulation by androgenic hormone. It also has the merit of explaining why almost all the pig-intergrades that have been described as possessing only degenerate testicular tissue possess a full complement of male accessory organs, whereas parts of the female reproductive tract are usually deficient. If the gonads had begun to develop as ovaries, the reverse relationship between the persistence of Müllerian and Wolffian structures would be expected. Indeed the descriptions of intersexual pigs which are available suggest, on the basis of our explanation, that the overlapping phases of development in male intergrades, as defined by Crew, have an order somewhat different from the one which he suggested, i.e. (1) differentiation of Wolffian ducts, (2) suppression of Müllerian ducts and (3) differentiation of the external genitalia. Such an order would explain those cases in which intergrades possessing a full complement of male accessory organs, but practically no trace of Müllerian structures, nevertheless have external genitalia of the female type. Presumably in such cases the alteration in the chemical environment of the developing foetus had set in after the Wolffian duct had differentiated

and the Müllerian duct had atrophied, but before the external genitalia had been fully moulded in a masculine manner. Furthermore, since deficiencies in the male accessory reproductive organs of intergrades appear to be mostly associated with the presence of ovotestes, it would seem that in such cases the transformation of the genically male gonads had begun sooner than in those intergrades which do not possess ovarian tissue, and before the differentiation of the Wolffian ducts had been properly achieved under the influence of the secretions of male differentiating gonads.

The hypothesis advanced here appears adequate to fit the anatomical facts about pig intergrades. It does not however assist in elucidating the anatomical problems involved in the secretion of oestrogenic hormone by degenerate testes. The responsibility for this anomaly cannot rest with the anterior lobe of the pituitary. The anterior lobe secretion, which in the female stimulates the growth of the follicles and the production of oestrogenic hormone, stimulates spermatogenesis in the male, while the hormone which is responsible for luteinization in the female activates the interstitial cells of the male. These cells, as not only our own observations but those of many previous investigators have shown, appear to be excessively active in the gonads of sex-intergrade pigs, and the inference is therefore that in intergrades the main gonadotrophic stimulation is that provided by the "luteinizing hormone". On the other hand the gonads in our intergrade were producing oestrogenic and not androgenic hormone. Clearly the reason for this anomalous activity must be some change in the gonads.

This problem resolves itself into two main issues. The first concerns the secretion by scrotal testes of oestrogenic hormone and the second the apparent implication of the cells of Leydig in this secretion.

It has been established that male and female gonadal hormones have ambisexual activity (see Korenchevsky, 1937, and Parkes, 1938, for reviews on this question) and furthermore that the male and female gonads can produce both male and female sex-hormones (see, for example, Callow & Parkes, 1936; Parkes, 1937; Lipschutz, 1937; Parkes, 1938; Fels & Diaz, 1938). It is clear, however, that the gonads normally produce an overwhelming preponderance of their homologous hormones, and that the heterologous action of the hormone produced is usually masked.

There is, however, evidence that the behaviour of the gonads is considerably influenced by the environment in which they are situated. Thus it is known that cryptorchidism is associated with sterility, and, as is pointed out by Moore (1939) in the most recent review of the problem, the scrotum functions as a thermo-regulator and provides a sufficiently cool environment for spermatogenesis to proceed. When the testes are subjected to a high temperature, as for example when they are spontaneously or experimentally retained in the abdomen, spermatogenesis ceases and the germinal epithelium rapidly degenerates until the testicular tissue is reduced to a mass of interstitial elements in which are embedded seminiferous tubules usually containing only a single layer of Sertoli cells.

Recent studies indicate that not only the spermatogenic function but also the endocrine function of the testes are affected by cryptorchidism and temperature. Thus observations on regressive changes in the accessory reproductive organs of experimentally cryptorchid animals suggest that the amount of male hormone secreted is diminished (see Moore, 1939), and this inference is borne out by Hanes & Hooker's (1937) finding, based upon the bio-assay of extracts, that cryptorchid testes contain less male hormone than do normal testes. Even clearer evidence about the influence of the environment upon the behaviour of the gonads comes from the work of Hill (1937*a*, *b*) and Hill & Strong (1938). These workers have shown that the involuted accessory reproductive organs of castrated male mice can be restored to normal by grafting ovaries into the ears. In view of the fact that restoration does not occur as a result of injection of either oestrone or progesterone, it can be inferred that the agent responsible for the change is some androgenic substance secreted by the transplanted ovaries. These, as indicated by growth of the mammary glands, secrete oestrogenic hormone as well. Experiment also shows that restoration of the accessory organs does not occur if the graft is made into the abdomen, and that it does not become apparent when the animals are kept at a high temperature. The temperature of the ear approximates to that of the scrotum, and Hill's general conclusion is that the ovaries secrete androgenic hormone if they are maintained at the temperature normal to the testes. All these results have been confirmed on rats by Deanesley (1938) who found, however, that ovarian grafts in the ears are androgenic whether or not the rats are kept at a low temperature, and that their simultaneous oestrogenic activity is manifested not only by changes in the mammary glands but also by fibro-muscular growth in the seminal vesicles.

If these observations are combined with the finding that the level of androgenic secretion is reduced in cryptorchid testes, it follows that the temperature of the scrotum provides the optimum condition for the production of androgenic hormone for both the testes and ovaries. Conversely it would appear that the optimum temperature for the secretion of oestrogenic hormone by the ovaries is that of the pelvic cavity. It is nevertheless clear that, although scrotal in position, the gonads of the intergrade we studied were secreting a preponderance of oestrogenic hormone. Similar gonadal activity (determined by bio-assay of urine extracts before and after castration) has been demonstrated in human male pseudo-hermaphrodites (Witschi, 1939), but with the exception of the stallion, there can be little doubt that relatively little oestrogenic hormone is normally produced by the mammalian testis when scrotal in position.

The problem becomes more complicated when it is remembered that the active tissue in intergrade testes appears to be the interstitial cells. Several lines of evidence suggest that these elements are normally responsible for the androgenic secretion of the testes (see Moore, 1939), even though it is also plain that cryptorchid testes, which appear to be abundantly supplied with

interstitial tissue, do not secrete as much androgenic hormone as do normal testes. It is this belief that in part underlies Willier & Witschi's conclusion (which is clearly not applicable to sex-intergrade pigs) that the degree of masculinization of secondary sex characters in free-martins is directly correlated with the "more or less testicular character" of the gonad.

If the interstitial cells were not responsible for the oestrogenic secretion of the intersexual gonads, the only other elements that could have been were the cells of Sertoli. Whether these cells have an internal secretory function is, however, highly doubtful. It is true that they are regarded as homologous with the granulosa cells of the ovary (Witschi, 1939), and that many observers believe that the granulosa is responsible for oestrogenic secretion in the female (but see below). On the other hand, the only observation we can find which suggests that the cells of Sertoli can produce oestrogenic hormone is the finding that dogs which have testicular tumours that involve these cells also often have prostates whose epithelium has undergone the widespread metaplasia that is associated with oestrogenic stimulation (Zuckerman & McKeown, 1938). (Such a prostatic change is not associated with pathological hyperplasia of the interstitial elements.) The cytological evidence also does not support the idea that the cells of Sertoli have an internal secretion. During normal spermatogenesis the neutral fat in these cells becomes transformed into lipoid which appears to pass into the spermatids (Hanes, 1911; Hanes & Rosenbloom, 1911). This transformation does not occur in the degenerated tubules of cryptorchid testes in which the cells of Sertoli, laden with neutral fat, are in any event reduced in numbers. In this connexion it should be remembered that cryptorchid testes are practically identical in appearance with the testes of sex-intergrade pigs (Hanes & Rosenbloom, 1911; Krediet, 1933).

In the circumstances it is reasonable to suppose that the tissues responsible for oestrogenic secretion in pig intergrade testes are the interstitial cells. According to Witschi (1939), who has recently reviewed the evidence, these cells most probably derive from the rete cords, which themselves originate from the mesonephric blastema, or according to some observers (see Willier, 1939) either from the germinal epithelium of the rete region of the genital ridge, or as outgrowths from the capsules of renal corpuscles. In the female frog the cords differentiate into "ovarial sacs", which later form the thecae of maturing egg follicles, and this relationship of theca and interstitial cells Witschi believes prevails among all tetrapod vertebrates. The derivation of such interstitial cells as are sometimes found in the ovaries of mammals from the theca interna of atretic follicles is also generally accepted (see Corner, 1932).

In view of this homology between ovarian theca and testicular interstitial cells, it is significant that the former appears to be, on the one hand, the main ovarian source of oestrogenic hormone (Corner, 1938), and on the other, responsible, when it has undergone luteinization, for androgenic secretion. Thus when androgenic activity of the ovary is elicited (e.g. in ear grafts,

after X-ray irradiation, after the administration of gonadotropic hormone; see Deanesley (1938) for references), it is associated with luteinization, and particularly with luteinization of the theca interna. Luteinization of the granulosa, according to Deanesley, does not lead to androgenic secretion. The female tissue which is homologous with the male interstitial cells is thus capable, in different phases, of both oestrogenic and androgenic secretion. The presumed secretion of oestrogenic hormone by the interstitial cells of the sex-intergrade testes thus becomes somewhat more understandable, even though the manner of their reversal of function remains entirely obscure.

SUMMARY

1. An experimental study has been made of an intersexual pig which possessed degenerate scrotal testes, vasa deferentia, vesiculae seminales (whose ducts ended blindly before opening into the urogenital sinus) and a prostate, as well as uterine horns of normal size, a corpus uteri and a vagina which opened into the urogenital sinus. The external genitalia were of the female type.

2. A laparotomy was performed at the start of the experiment, and a specimen of uterus and vas removed. A piece of testis was also excised. Starting 3 months later the animal was given a $2\frac{1}{2}$ months' course of male-hormone injections, at the end of which further specimens of uterus, vas and testis were removed. It was then injected for $2\frac{1}{2}$ months with oestrogenic hormone, and again specimens of the reproductive organs were obtained. Four months later the animal was autopsied.

3. Histological study showed that the uterus at the start of the experiment was identical in structure with the specimen removed at the end of oestrogenic treatment, and altogether different from the specimens removed after androgenic treatment and after castration. The latter two specimens differed in detail from each other, but both were greatly involuted.

4. The vas at the start of the experiment was identical in appearance with the specimen removed after oestrogenic treatment, and altogether different from the activated specimen removed after androgenic treatment. It was also completely different from the very involuted piece removed after castration.

5. The gonads consisted of degenerate seminiferous tubules, containing only Sertoli cells, set in a matrix of swollen interstitial cells. The general appearance of the tissue remained unaltered in the various phases of the experiment, but changes occurred in the proportions of the interstitial cells. These enlarged significantly under the influence of oestrogenic treatment, and the nuclear-cytoplasmic ratio decreased significantly. No change occurred in cell size as a result of androgenic stimulation, but the nuclear-cytoplasmic ratio showed an increase which was almost significant statistically.

6. It is concluded from these observations that before the start of the experiment the gonads were secreting only, or a preponderance of, oestrogenic hormone.

7. Current theories of the morphogenesis of intersexuality in pigs appear inadequate to fit these facts. The alternative hypothesis is suggested that pig sex-intergrades are genetic males whose differentiation was altered as a result of a change in their chemical environment following upon the passage of an excessive amount of maternal sex-hormone across the placenta. The secretion of oestrogenic hormone by male gonads is regarded as a quasi-permanent functional reversal, which, had it occurred earlier in development, may have led to the differentiation of a cortical cap of ovarian tissue. On this theory, which, with qualifications, is not dissimilar from a hypothesis that has been advanced to explain human male intersexuality, it is possible to interpret variations in the differentiation of the Wolffian and Müllerian ducts, and the urogenital sinus.

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EXPLANATION OF PLATES I–IV

Plate I

Fig. 1. Uterus at start of experiment, 3 February 1937. ×6·6.
Fig. 2. Uterus after phase of oestrogenic stimulation, 8 November 1937. ×6·9.
Fig. 3. Uterus after phase of androgenic stimulation, 4 August 1937. ×10·4.
Fig. 4. Uterus, after castration, 3 March 1938. ×8·5.
Fig. 5. Vaginal epithelium and gland, after castration, 3 March 1938. ×344.

Plate II

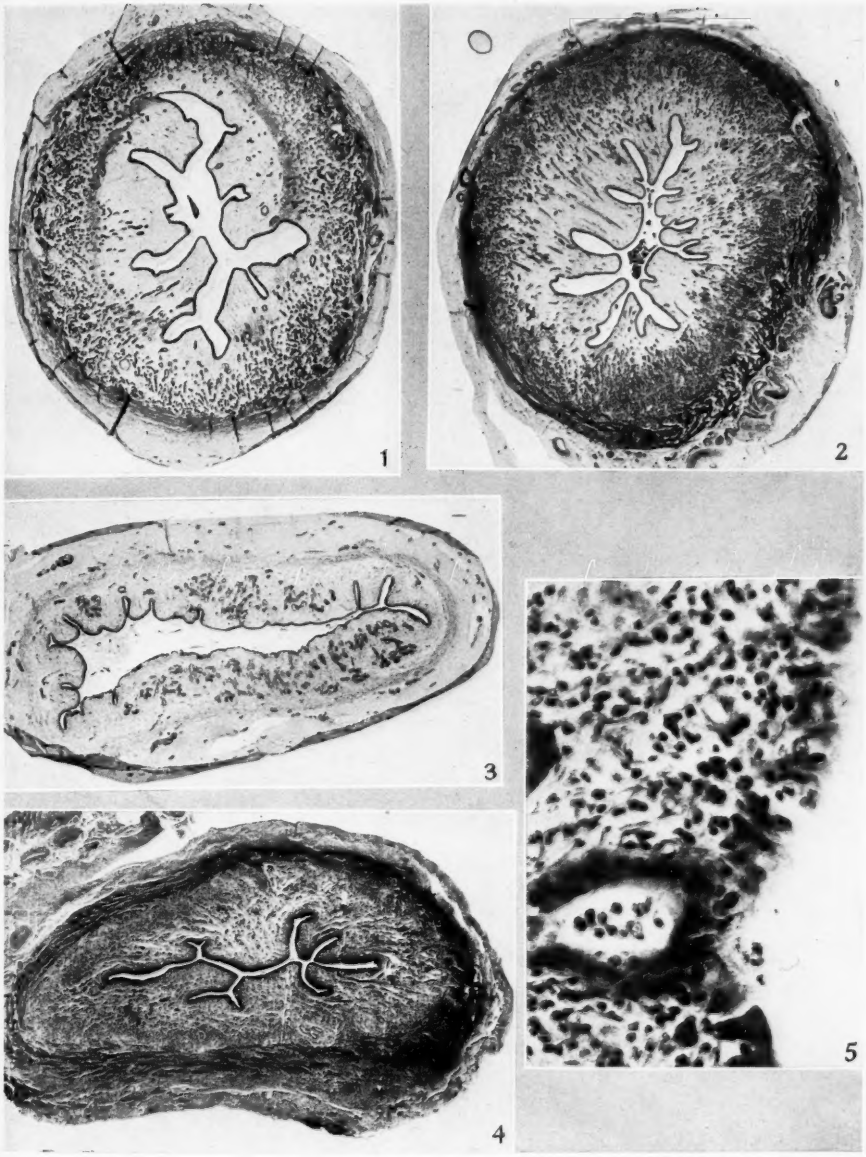
Fig. 6. Endometrial glands at start of experiment, 3 February 1937. ×260.
Fig. 7. Endometrial glands after phase of oestrogenic stimulation, 8 November 1937. ×260.
Fig. 8. Endometrial glands after phase of androgenic stimulation, 4 August 1937. ×260.
Fig. 9. Endometrial glands after castration, 3 March 1938. ×260.
Fig. 10. Epithelium of cavum uteri at start of experiment, 3 February 1937. Note dividing cells. ×600.
Fig. 11. Epithelium of cavum uteri after phase of oestrogenic stimulation, 8 November 1937. Note mitotic figure. ×600.
Fig. 12. Epithelium of cavum uteri after phase of androgenic stimulation, 4 August 1937. ×600.
Fig. 13. Epithelium of cavum uteri after castration, 3 March 1938. ×600.

Plate III

Fig. 14. Vas deferens at start of experiment, 3 February 1937. ×93.
Fig. 15. Vas deferens after phase of oestrogenic stimulation, 8 November 1937. ×93.
Fig. 16. Epithelium of vas deferens after phase of androgenic stimulation, 4 August 1937. ×93.
Fig. 17. Vas deferens after castration, 3 March 1938. ×93.
Fig. 18. Prostatic glands and collecting duct after castration, 3 March 1938. ×250.
Fig. 19. Epididymis after phase of oestrogenic stimulation, 8 November 1937. ×30.

Plate IV

Fig. 20. Corpus uteri, with the vasa deferentia on its ventral surface. Non-glandular polypoid can be seen within the uterus. ×7.
Fig. 21. Vas deferens passing into vesicula seminalis. ×6·6.
Fig. 22. Low stratified epithelium of urogenital sinus after castration, 3 March 1938. ×370.
Fig. 23. Transverse section of duct of vesicula seminalis near its termination and lying to the side of (below in figure) the lumen of the glandular vagina. ×50.
Fig. 24. Section of testis, 8 November 1937, showing cells of Sertoli in two "seminiferous" tubules, and abundant interstitial tissue. ×220.
Fig. 25. Vesicula seminalis after castration, 3 March 1938. ×250.



Figs. 1–5.

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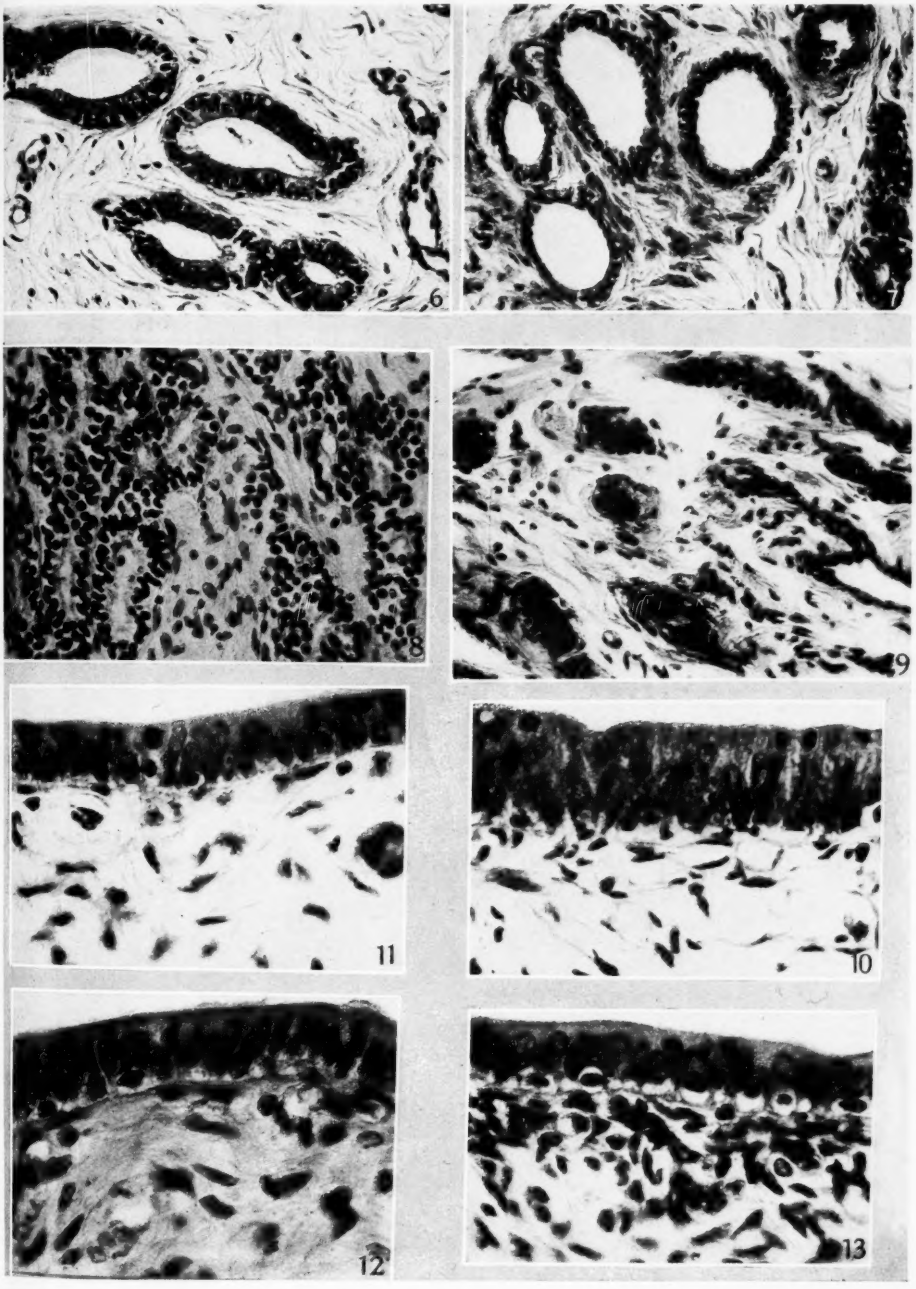

Figs. 6–13.

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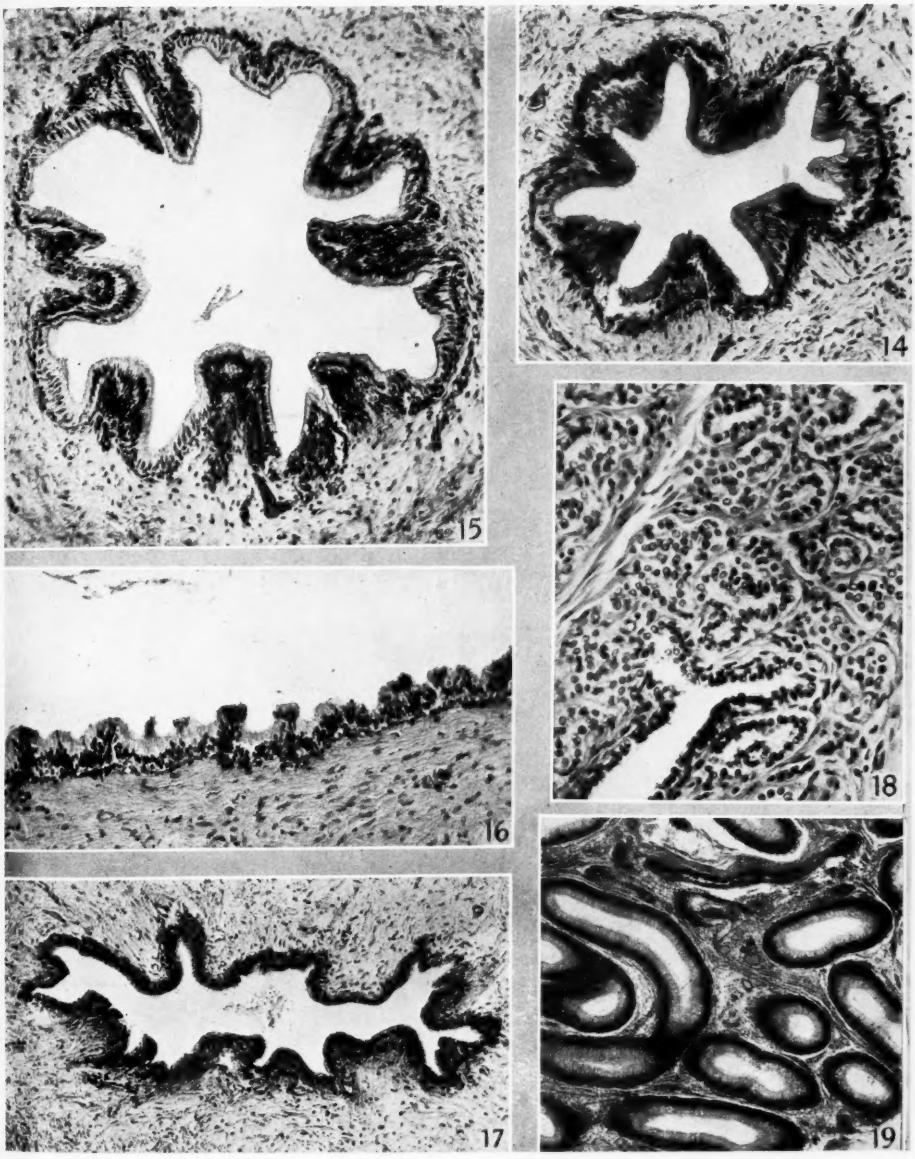

Figs. 14–19.

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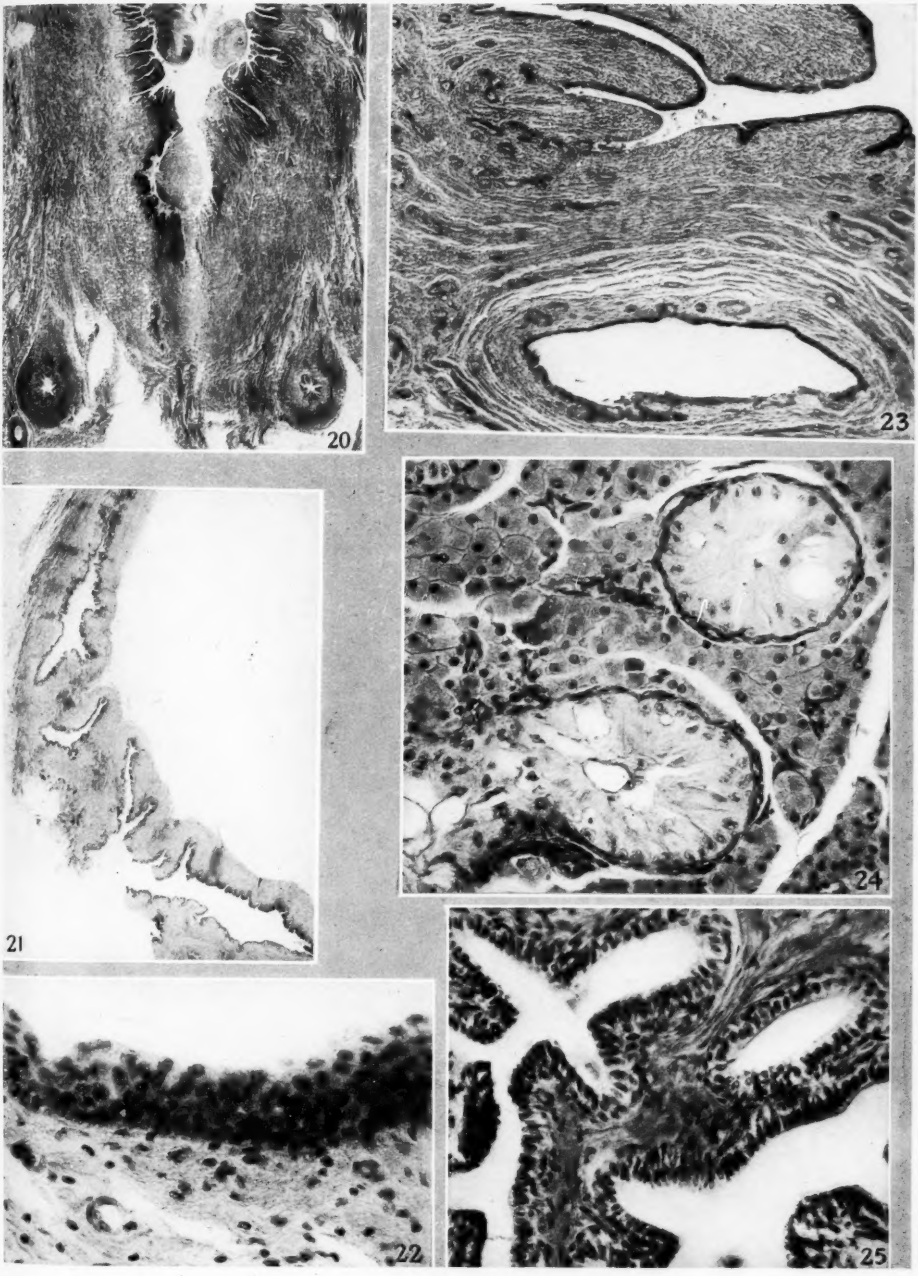

Figs. 20–25.

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THE PROJECTION OF THE CEREBRAL CORTEX ON THE PONS AND CEREBELLUM IN THE MACAQUE MONKEY

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It is well known that the cerebral hemispheres and cerebellum are connected anatomically by means of cortico-ponto-cerebellar tracts, and that the pontine nucleus plays a role of considerable importance as an intermediate relay station in this pathway. There is as yet, however, no agreement of opinion concerning either the precise origin of the cortico-pontine tracts and the site of their termination in the pontine nucleus, or the manner in which the latter projects on to the cerebellar cortex. This paper is the report of an experimental investigation undertaken to study the manner in which the cerebral and cerebellar cortex are associated, and to determine the part played by the pontine nucleus in this relationship.

The investigation was divided into three parts. The first was devoted to a study of the normal cyto-architecture of the pontine nucleus of the macaque monkey; in the second, the origin and termination of the cortico-pontine system were determined by tracing, by means of the Marchi method, the degeneration following localized cortical lesions, while in the third the ponto-cerebellar system was investigated by examining the retrograde degeneration occurring in the pons after lesions restricted to the cerebellar cortex. By correlating the observations made in this way it was hoped to be able to establish a more accurate relationship between the cerebral and cerebellar cortex.

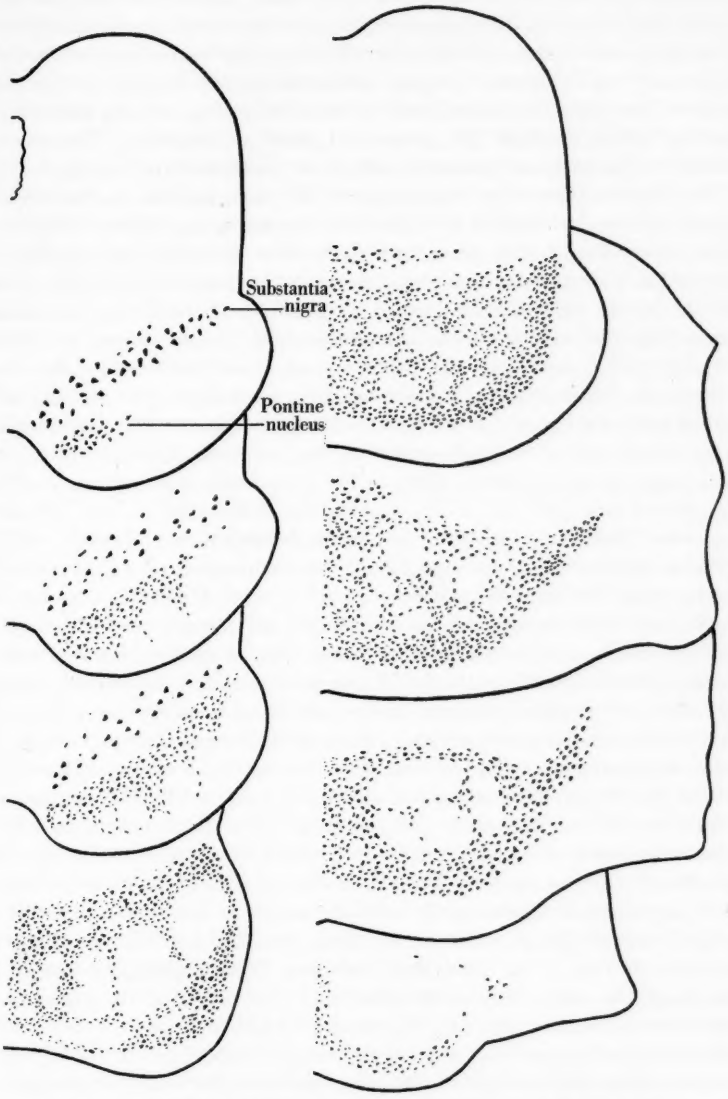
I. THE HISTOLOGICAL STRUCTURE OF THE PONTINE NUCLEUS

The observations were made on sections of the pons stained by the Nissl and Bodian methods. Transverse blocks of a suitable size were cut and placed in 70% alcohol for 24 hr. and then dehydrated through 5% acetic acid in absolute alcohol. After clearing in cedar wood oil they were embedded in paraffin and sections cut at 15μ. Alternate sections were stained by Nissl's method and by the method of Bodian. For the Nissl method the sections were stained with 1 in 5 Borrel's methylene blue at 58° C., differentiated in Gothard's solution and mounted in neutral balsam.

The pontine nucleus is not strictly localized to the pons as the latter is defined topographically. It extends into the lower part of the mid-brain above, and for a short distance into the upper part of the medulla below. In the mid-brain the nucleus is situated in the peduncular region immediately ventral to

the substantia nigra, in the pons it is distributed extensively over its ventral half, while in the medulla it is arranged as a collar of cells about the issuing pyramidal tract.

The nucleus is first seen in transverse sections through the mid-brain at the level of the summit of the inferior colliculus where it appears as a small condensed mass of cells situated in the cerebral peduncle just ventral to the medial end of the substantia nigra. This nuclear mass undergoes certain characteristic changes as it is traced distally. First it increases in size and flattens out to form a band which extends laterally across the peduncle, and then becomes thicker as the nucleus is traced distally. The dorsal margin of this band remains straight, but by the time the nucleus is stretching about half-way across the peduncle the ventral margin becomes serrated by the appearance of irregular spurs of cells which are directed ventrally, and which distally become the intrapeduncular strands. Before the upper border of the pons is reached the nuclear band extends to the lateral margin of the peduncle, and is thick and markedly serrated ventrally, while it shows additional thickenings at the medial and lateral ends. By the continued addition of cells ventrally to the serrations, cell rings are outlined, and in this way the fibre mass ventral to the nuclear band becomes subdivided into a number of reticular columns. With a further increase of cells more of these columns so outlined are added, so that by the time the pons is reached the pontine nucleus consists of a relatively broad base of cells stretching across the pons, ventral to, and continuous with, which is a mass of reticular columns which reaches as far forwards as the ventral border of the pons. This arrangement persists until the distal border of the pons is approached when the central columns start to blend by the disappearance of the net-like intrapeduncular cell strands. In this way larger columns are outlined around which are arranged smaller satellite columns. This process of blending continues until at the distal border of the pons there is one large central column, the pyramidal tract, which carries a collar of the pontine nucleus with it into the medulla for a very short distance. This collar is gradually lost, first dorsally, then laterally, then medially and finally ventrally. As the main nuclear mass is traced distally additional changes are observed. About midway down there is a sudden thickening of the ventral element of the peripeduncular cell ring which first appears ventrolaterally. The ventral element continues to increase in size until the distal border of the pons is reached when it abruptly diminishes in size, and follows the ventral aspect of the pyramidal tract for a short distance into the medulla as an arch-shaped nucleus. In the distal half of the pons a thin part of the basal or dorsal portion of the nucleus becomes separated off dorsally by an ill-defined set of transverse fibres. Both these parts of the basal aspect of the nucleus gradually disappear as the nucleus is traced distally, the dorsal terminating before the ventral. There are two extensions from the parent nucleus. Towards the middle of the pons a triangular process of cells separates abruptly from the dorsal surface of the thick medial end of the basal portion of the nucleus, and extends laterally from the mid-line



Text-fig. 1. Diagrams illustrating the various cyto-architectural phases through which the pontine nucleus passes as it is traced distally.

half-way across the pons. Distally it becomes shorter and thicker until it terminates towards the distal border of the pons. About half-way down the pons the lateral part of the nucleus originally derived from the thickened lateral aspect of the base sends a process laterally from which groups of cells are shed progressively as the pontine grey is traced still further distally. At the distal border of the pons this lateral horn is completely lost, and the pontine grey becomes arranged about the pyramidal tract as described. The essential features of this cyto-architectural pattern are illustrated in Text-fig. 1.

Throughout there is no separation of the main nucleus, as has been described, into separate and distinct elements; it remains as a diffuse cellular mass which shows thickenings in certain parts. The nomenclature employed to describe the various parts of the parent mass is that used by Borowiecki (1911), Masuda (1914), and Winkler (1927). The part of the cell ring immediately surrounding the reticular columns, together with the interlacing cell strands outlining them, represent the peripeduncular and intrapeduncular nuclei respectively, which together form the peduncular nucleus. The identity of the original basal nucleus is difficult because it might represent the dorsal nucleus, or the dorsal part of the peduncular nucleus, or both. From the fact that it gives origin to the serrations forming the intrapeduncular nucleus ventrally, part of it at least probably corresponds to the peduncular nucleus. Distally a transverse fibre band separates off an ill-defined dorsal element which is probably the true dorsal nucleus. Under the circumstances it would perhaps be best to regard the basal element as representing a combined dorsal nucleus and dorsal part of the peduncular nucleus, which are normally separated by the stratum complexum dorsale in some forms. The triangular nucleus described, which separates away from the dorsal element dorsally, is the reticular nucleus. The thickened medial and lateral ends of the basal band represent the medial and dorso-lateral nuclei respectively, while the thickened ventral portion of the peduncular nucleus is a combination of the true ventral nucleus and the ventral part of the peripeduncular nucleus which are separated by the stratum complexum ventrale in the rabbit, cat, and man. The thick lateral part of the peduncular nucleus, which reaches its greatest development distally, corresponds to the lateral nucleus. It must be stressed that in the macaque monkey these nuclei run indeterminately into one another, and that no specific and isolated nuclear groups exist, as has been described by Winkler (1927) and others in the case of the rabbit, cat, and man. Winkler (1927) has stated that the size of the cells varies in the different nuclei, and that the nuclei can be differentiated by the type of cell present. In the macaque monkey no such differentiation is possible. Large, medium-sized, small and very small cells are scattered diffusely throughout the entire nucleus. The main features may be summarized as follows:

(1) The ventral nucleus is large and is best developed in the distal half of the pons. No stratum complexum ventrale is present, so that this nucleus is blended throughout with the ventral part of the peduncular nucleus.

(2) The lateral nucleus is also large and shows its greatest development in the distal half of the pons.

(3) The peduncular nucleus is well developed throughout.

(4) The medial and dorsal nuclei are poorly developed. They reach their maximum size in the rostral half of the pons.

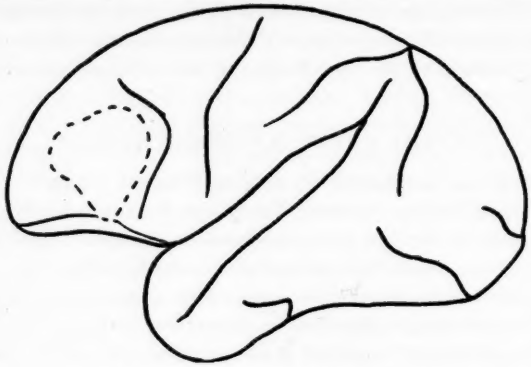
Three fibre pathways which are believed to terminate in the pons have been described in the cerebral peduncle. According to Winkler (1927) the fronto-pontine tract terminates chiefly in the rostral third of the pons about the dorsal nucleus and associated dorsal element of the peduncular nucleus, which are best developed in this region; the parieto-pontine tract ends chiefly in the distal third of the pons about the ventral, medial, and intrapeduncular parts of the peduncular nucleus, and also about the dorso-lateral and ventral nuclei, while the temporo-pontine tract breaks up in the middle and distal thirds, chiefly in association with the lateral nucleus which reaches its greatest development in that region. The structure of the nucleus in the macaque monkey favours a similar arrangement. The second part of this paper deals with an experimental study of this problem of the termination of the cortico-pontine tracts in the pons.

II. THE CORTICO-PONTINE SYSTEM

The observations are based on six experiments in each of which the ordinary macaque monkey was used. The lesions were quite localized and small, and were designed to involve, as far as possible, not more than one or two of Brodmann's cortical areas. The method of devascularization was used since it is the best method of destroying the cortex with a minimum of damage to the subjacent white matter and fibre tracts. A previous investigation (Sunderland, 1938) has shown that the cerebral cortex receives its entire effective blood supply from superficial pial vessels, and that these vessels also supply the subjacent white matter to a limited degree, so that, if the blood supply to any cortical area is interfered with, the area is completely destroyed. The degree to which the subjacent white matter is involved varies, but in any case it is no more extensive than would be produced by actual removal of brain tissue with a knife or diathermy, because cortical ablation must necessarily interfere with the associated blood vessels. The method of devascularization is preferable because it is simple, it does not involve instrumental interference with the cortex and is just as effective as cortical ablation.

Anaesthesia was induced by the intraperitoneal injection of sodium ethyl barbiturate (nembutal) using 0·7 c.c. of a 5 % solution per kilogram of body weight. The cerebral hemisphere was exposed by turning down a rectangular osteoplastic flap and the lesion produced as described. All bleeding was carefully controlled before closing the wound. The deep structures were brought together with interrupted linen sutures and the skin wound closed with a continuous subcuticular stitch. The wound was not dressed. The post-operative treatment was to keep the animal warm. Recovery in all cases was rapid and

uneventful, the animals being alert and active within 24 hr. All the wounds healed well. The animals were allowed to survive for 17 days and were then killed. The vascular system was washed clear with saline by way of the aorta, and then perfused with 10 % formalin. The brain was removed one hour later and washed in running water for one hour. The mid-brain, pons, and medulla were then removed *en masse* and the block divided into sagittal slices. The blocks were mordanted in potassium bichromate, and then passed through potassium bichromate containing varying quantities of osmic acid, cleared in spirit and embedded in celloidin. Sections were cut at 15μ and every fifth examined for fibres of degeneration. In tracing tracts of degeneration in this way the limitations of the Marchi method have been taken into account. For purposes of control both sides of the brain-stem were sectioned and examined. All the lesions were carefully examined, measured and drawn. The observation that devascularization results in complete cortical destruction with only a



Text-fig. 2. The cortical lesion in Exp. 1.

limited involvement of the immediately subjacent white matter was confirmed in every case.

The experiments

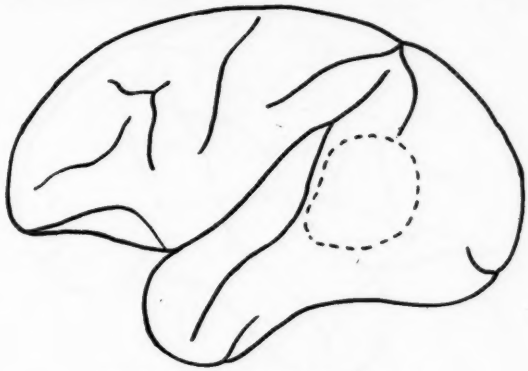
Exp. 1. Monkey 54A. Operation 5 May 1938. Killed 23 May 1938. The lesion was situated in the prefrontal region and involved most of area 8, encroaching on areas 9 and 10. It measured 17 mm. coronally, and 15 mm. sagittally. The anterior margin was 12 mm. from the frontal pole, while its posterior margin traced a parallel course 3 mm. anterior to the sulcus arcuatus. Inferiorly it just reached the lower margin of the brain (Text-fig. 2).

Histological examination. There were a few Marchi granules scattered in an irregular manner in the vicinity of the substantia nigra, in the outer part of the rostral portion of the pons, and in the medulla along the course of a few fibres of the pyramidal tract. The deposit was an irregular one and there was no gathering of granules in a linear pattern suggesting tract degeneration. In this

series there was no evidence indicating degeneration in any specific pathway in the mid-brain, pons, or medulla. The little deposit present was located on the same side as the lesion.

Exp. 2. Monkey 46A. Operation 29 June 1938. Killed 16 July 1938. The lesion was situated at the lower end of the upper limb of the sulcus lunatus and involved parts of areas 17, 18 and 19. It measured 18 mm. antero-posteriorly, and 14 mm. in a vertical direction. Anteriorly the margin reached close to the temporal sulcus, while it was 24 mm. from the occipital pole posteriorly and 7 mm. from the lower border of the brain inferiorly (Text-fig. 3).

Histological examination. The degeneration outlined in this series was very similar to that which will be described in the case of Exps. 3 and 6, except that the granular deposit was not so great. There was a precipitate of osmic granules in the outer third of the peduncle, and in the pons the distribution of the deposit showed that fibres coming from that portion of the peduncle were ter-



Text-fig. 3. The cortical lesion in Exp. 2.

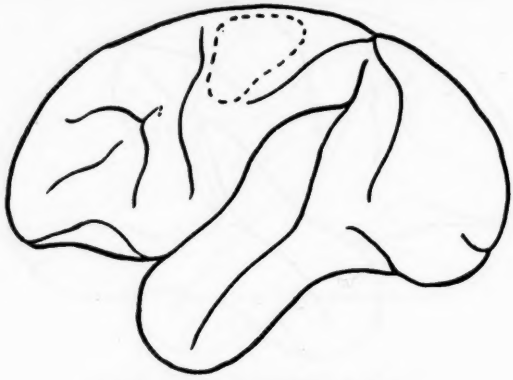
minating in the vicinity of the ventro-lateral portion of the nucleus in the rostral half of the pons. The more medially situated of the involved fibres in the outer third of the peduncle pursued a course through the pons dorsal to the others to reach the medulla.

Exp. 3. Monkey 47A. Operation 24 June 1938. Killed 13 July 1938. The lesion involved areas 5, 2 and 1 in the upper part of the post-central gyrus. It measured 17·5 mm. sagittally and 13 mm. coronally. The upper margin of the lesion was 5 mm. from the superior border of the brain. Anteriorly the lesion just reached the sulcus centralis while posteriorly it extended along the intra-parietal sulcus. Neither sulcus was damaged (Text-fig. 4).

Histological examination. In the mid-brain there was a marked degeneration of the fibres in the lateral half of the peduncle. These could be traced into the pons and the arrangement of the Marchi granules indicated that the lateral fibres were breaking up in the neighbourhood of the lateral, ventral, and intra-peduncular elements of the pontine nucleus in its rostral three-fourths, and

chiefly towards the caudal end of this region. The more centrally situated pathways showing a deposit continued through the pons into the medulla, where a dense deposit indicated that many degenerated fibres were entering this region. It may be noted at this stage that the degenerated fibres in this series appeared to terminate more distally than those in the occipital and temporal lesions. These changes were unilateral, and the observations indicate that parieto-pontine fibres are present in the outer part of the foot of the peduncle, and that they are distributed over the rostral three-fourths of the pontine nucleus, chiefly caudally, in association with the ventral, lateral, and intrapeduncular parts of the pontine nucleus.

Exp. 4. Monkey 49A. Operation 12 May 1938. Killed 30 May 1938. The lesion was an extensive one involving most of the outer surface of the occipital lobe. It measured 28 mm. coronally and 14 mm. antero-posteriorly. The margin of the lesion was 15 mm. from the occipital pole posteriorly, and 5 and 3 mm.

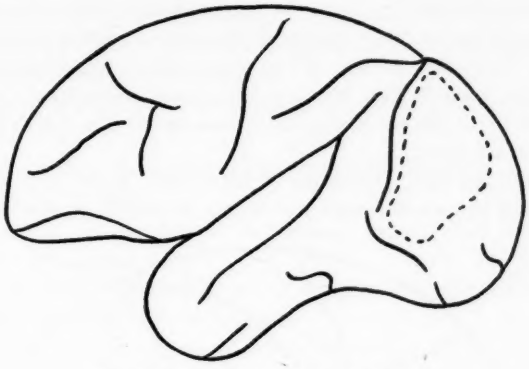


Text-fig. 4. The cortical lesion in Exp. 3.

from the inferior and superior borders of the brain respectively. Anteriorly it followed the sulcus lunatus, but remained separated from it by a distance of 1 mm. The lesion involved area 17 and possibly the immediately adjacent margin of area 18 (Text-fig. 5).

Histological examination. In the mid-brain there was a heavy deposit of large Marchi granules in the outer half of the peduncle which was more marked laterally. There was also some deposit in the tegmentum. The deposit in the peduncle could be traced into the pons. Laterally the arrangement of the granules suggested the termination of fibres in the rostral half of the pons about the lateral and ventral parts of the pontine nucleus. The pattern was very similar to that recorded in Exp. 6 except that the deposit was less dense. Medially, however, degenerated fibres, clearly outlined by a linear deposit, could be traced into the medulla. Fine granules were also scattered through the inferior cerebellar peduncle and the trapezium. There was no deposit on the

contralateral side. The precipitate in this series was less dense than in Exps. 2, 3 and 6, and considering that the cortical lesion in this case was larger than in the others the suggestion is that the contribution of the occipital cortex to a cortico-pontine system is less than that of the other areas. The evidence does indicate, however, that fibres arising in the occipital lobe reach and terminate in the pons.



Text-fig. 5. The cortical lesion in Exp. 4.

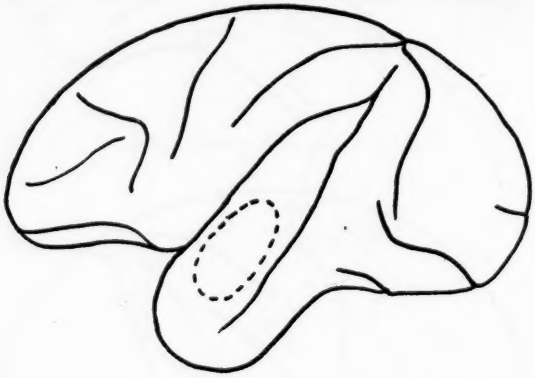
Text-fig. 6. The cortical lesion in Exp. 5.

Exp. 5. Monkey 50A. Operation 24 May 1938. Killed 10 June 1938. The lesion was located in the prefrontal region above the sulcus arcuatus and involved areas 6 and 9. Its dimensions were 19 mm. antero-posteriorly with a maximum coronal diameter of 12 mm. Antero-inferiorly the margin of the lesion was in contact with the sulcus rectus while superiorly it reached a distance of 5 mm. from the superior border of the hemisphere (Text-fig. 6).

Histological examination. The outer two-thirds of the cerebral peduncle was free of osmic deposit. The inner one-third showed a dense granular speckling

which continued into the pons. The nature of the deposit in the lower part of the mid-brain medially, and in the rostral half of the pons in the neighbourhood of the dorsal and associated intrapeduncular elements of the pontine nucleus, suggests that most of the fibres terminate there. Other fibres showing a much finer precipitate could be traced through the pons into the medulla where degeneration was limited to the appearance of a few granules only. Most of the degenerated fibres evidently end in the pons and do not reach the medulla. The sections indicate that cortico-pontine fibres arise in areas 6 or 9, or both, and terminate in the dorsal portion of the pontine nucleus, chiefly in its rostral third. Since the lesion in Exp. 1 involved area 9 with no resultant degeneration, it may be taken that the cortico-pontine fibres revealed in this experiment take origin from area 6 only.

Exp. 6. Monkey 52A. Operation 26 May 1938. Killed 13 June 1938. This lesion measured 16 mm. in length and 10 mm. in width. It was situated in the



Text-fig. 7. The cortical lesion in Exp. 6.

inferior portion of the superior temporal gyrus, but did not involve the Sylvian fissure or temporal sulcus. It was localized to area 22 (Text-fig. 7).

Histological examination. Once again there was a dense Marchi precipitate in the outer half of the peduncle and in the pons. In the rostral half of the pons the arrangement of the granules indicates that the more lateral fibres terminate about the ventral and lateral aspects of the nucleus above, and about the lateral part below. The most lateral fibres are seen swinging ventrally at the upper border of the pons to their termination. The degenerated fibres situated more medially take a course in the pons dorsal to those already described and pass on to enter the medulla. There was a similar deposit on the contralateral side which, though similar in distribution, was much less dense than on the ipsilateral side. The bilateral nature of the change and the fact that many degenerated fibres pass on to enter the medulla make it difficult to assess the site of termination of the temporo-pontine tract in this particular case because the precipitate,

situated more deeply in the fibres which are destined to reach the medulla, tends to obscure the exact site of termination of the temporo-pontine fibres which are situated more laterally. However, a superposition of sections shows that the fibres appearing in the distal half of the pons pass on to reach the medulla. Taking into consideration these findings and the similarity they bear to those recorded in the other lesions involving the occipital and temporal regions, it may be inferred that fibres do arise from this cortical area which reach the rostral half of the pons and terminate there, chiefly about the ventral and lateral parts of the pontine nucleus.

The following conclusions may be drawn from an analysis of these observations:

(1) Cortico-pontine tracts take origin in the frontal, parietal, occipital, and temporal lobes of the brain.

(2) Correlating the amount of the deposit present and the size of the lesions, it may be inferred that:

(i) Areas 8 and 9 probably do not contribute to this system.

(ii) Area 6 sends fibres to the pons.

(iii) The contribution from the frontal areas studied is less than that from any areas of corresponding size in the parieto-occipito-temporal region. So far, then, as the regions investigated are concerned, it would seem that in the monkey the cortico-pontine pathway occupying the lateral segment of the peduncle is of more significance than that occupying the medial, if the number of fibres alone be accepted as a criterion of importance, and such a relationship is reflected in the structure of the pontine nucleus. The ventral and ventro-lateral parts of the nucleus, which are associated principally with the parieto-occipito-temporal pathway, are large, while the dorsal element about which the fronto-pontine tract appears to terminate is poorly developed.

(iv) The contributions from the parietal and temporal areas studied appear to be of equal significance, but each exceeds that coming from the occipital areas.

(v) Areas 18 or 19, or both, send more fibres to the pons than area 17, for the deposit in Exp. 2, which involved parts of areas 17, 18 and 19, greatly exceeded that recorded in Exp. 4 where the lesion was larger and was almost entirely limited to area 17.

(3) In the peduncle the frontal fibres occupy the medial third while the remainder are all localized to the lateral half.

(4) In the pons all the cortico-pontine fibres revealed by this study terminate ipsilaterally, in approximately the rostral three-fourths of the pons.

(5) The fronto-pontine fibres appear to terminate about the dorsal part of the pontine nucleus chiefly, while all the others end in relationship with the ventro-lateral and lateral portions of the nucleus. The frontal fibres terminate more rostrally, and the parietal more distally than the remainder, though there is a considerable overlap of all systems in the intermediate zones.

Discussion

Fronto-pontine tract. It is now well established that cortico-pontine fibres take origin in the frontal lobe, but there is no conclusion concerning the particular fields from which they arise. Monakow (1895, 1905), Mingazinni (1895), and Bechterew (1900) placed the origin in the first and second frontal convolutions. From a study of pathological material in man Dejerine (1901) came to the conclusion that the tract took origin in the opercular gyrus and in the foot of the third frontal convolution, while Flechsig (1903), by the same method, localized the origin to the pre-rolandic gyrus. Quensel (quoted from Edinger, 1900), on the other hand, located the origin in that portion of the frontal lobe situated just in front of the genu of the corpus callosum on the medial surface of the brain, while Winkleman & Eckel (1929), Rutishauser (1899), and Ferrier & Turner (1898) have suggested that the area of origin is situated further forwards in the frontal part of the lobe. Mettler (1935) believes that the pons receives fibres from area 9 but not from areas 4 or 6. On the contrary, Pfeiffer's myelogenetic studies (1934) indicate that the system takes origin from the lower part of area 6, and this is supported by the work of Levin (1936) who has shown that in the monkey there is a cortico-pontine tract arising from areas 4 and 6 which occupies the middle segment of the peduncle, while concerning Arnold's bundle (which is usually regarded as the fronto-pontine tract and which occupies the inner segment of the peduncle) he writes "the precise area has not been clearly demonstrated but it appears here also to be the inferior frontal gyrus". The results of this present study show that a fronto-pontine tract, or portion of it, definitely takes origin from area 6, and that this tract occupies chiefly the medial segment of the peduncle. This observation agrees with those of Pfeiffer and Levin, except that the course taken by the tract does not coincide with that reported by Levin. So far as the other frontal fields are concerned it may be stated that area 8 did not give any contribution to this system and that it is unlikely that areas 9 and 10 do so either. However, the evidence for an origin further forwards in the prefrontal region appears to have been confirmed by the experimental work of Ferrier and Turner.

Borowiecki (1911), Masuda (1914), Jakob (1928), Bechterew (1900), Levin (1936), Abbie (1934), and Winkler (1927) have all produced evidence to show that this pathway terminates in the rostral half of the pons. Spitzer & Karplus (1907) alone carry the tract into the distal half. The results of this study confirm the general impression of a localization to the rostral half of the pons.

Concerning the precise nuclear groups with which this pathway comes into relationship, Winkler (1927) has written that the termination is chiefly about the dorsal, lateral, and adjacent peduncular elements, while Levin (1936) names the dorsal pontine nucleus for the fronto-pontine tract originating in areas 4 and 6, and the substantia nigra, dorso-medial nucleus and the adjacent part of the tegmentum for Arnold's Bundle. The results of the present study, with the reservations already cited, confirm these observations, while they disagree with

those of Bechterew (1900), who reported that this tract ends in relationship with the ventral and ventro-medial nuclei.

Other cortico-pontine systems. In 1851 Türck described a tract, since called Türck's Bundle, in the lateral part of the cerebral peduncle. Following this discovery, interest in the origin and termination of the component fibres in this tract has stimulated much research, but, as yet, there is no agreement of opinion regarding these details. The work of Meynert (1867), Brissaud (1881), Ballet (1881), and Charcot (quoted in Marie & Guillain, 1903), who regarded this tract as a cortical afferent system, need not be discussed. A cortical origin was proved conclusively and independently by Bechterew (1900), Dejerine (1893), and Kam (1895), and was later confirmed by the experimental studies of Ferrier & Turner (1898) on monkeys, and Pusateri (1898) on cats. As the chief difficulty lies in the confusion which exists concerning the precise cortical areas giving origin to the fibres of this pathway, it will be convenient at this stage to refer briefly to the relevant literature dealing with the problem.

There is abundant evidence to show that at least some fibres arise in the temporal lobe. Dejerine (1893, 1901), from a detailed study of pathological material, concluded that this tract takes origin from the caudal parts of the second and third temporal convolutions. Marie & Guillain (1903), from a parallel study, claimed that most of the fibres come from the third convolution, with some possibly coming from the first and second; they were convinced that none come from the occipital and parietal lobes. Ferrier & Turner (1898), and Mettler (1935) have confirmed a temporal origin experimentally. Following lesions of the first temporal convolution in monkeys the former found degeneration in this tract, while Mettler, as the result of degeneration experiments, has demonstrated that the middle and inferior temporal gyri give origin to cortico-pontine fibres in monkeys, and that few, if any, come from the superior temporal region. In a later paper, however, Mettler (1935) has written that fibres do arise from the upper part of the superior temporal gyrus. Additional evidence in favour of a temporal origin is expressed in the work of Kam (1895), Winkleman & Eckel (1929), Brodmann (1910), Kattwinkle & Neumayer (1910). Winkler (1927) reports the presence of separate temporo-pontine and parieto-pontine pathways, and that the latter occupies a situation medial to the other in the middle third of the peduncle. Others to describe parietal fibres amongst the temporal are Sioli (1889), Rossolymo (1886), Fryliusk (1889), and Monakow (1905). Gerwer (1899), working in Bechterew's laboratory, found that occipito-pontine and temporo-pontine fibres were revealed by the descending degeneration following cortical lesions in the dog. The former were outlined by a small accessory deposit, the latter by a main gross deposit. Thus, on the basis of experimental and pathological studies the case for the presence of temporal fibres seems to have been definitely proved. Flechsig (1903), Rundles & Papez (1938), Mills & Spiller (1896), Rhein (1911), and Löwenstein (1911) have produced some evidence to the contrary, but this is inconclusive. Flechsig's criticisms, based on a study of tract degeneration in pathological material, were directed only

against Dejerine's conclusions. Mills & Spiller (one case of cerebral abscess), Löwenstein (one case of cerebral tumour) and Rhein (two cerebro-vascular lesions and one case of cerebral tumour) based their observations on clinical material in which a diffuse pathological process was responsible for the lesion, and information so derived can never be accepted as conclusive. Rundles & Papez (1938) have recorded the observation that, following temporal lobectomy in the mangabey and baboon, the outer part of the peduncle remains unchanged, and they have claimed that almost three-fourths of the peduncle is made up of fibres taking origin in the frontal lobe, while the remainder come from the post-central and parietal areas. This experimental work is the only acceptable evidence against a temporal origin for the tract. However, it would seem that such an isolated finding is insufficient proof against the convincing and abundant evidence presented in favour of such an origin.

Investigators, other than those already mentioned, who have recorded the presence of parietal fibres in the lateral part of the peduncle are Le Gros Clark & Boggon (1935), Biemond (1930), Minkowski (1923, 1924), and Mettler (1935), while Jelgersma (1887) and Brero (1897) believed that this pathway was exclusively parietal in origin. The only evidence opposed to these experimental observations is that of Marie & Guillain (1903) which was based on a study of pathological material, so that, considering the relative merits of the conflicting views, it is most probable that a parietal contribution does exist.

There is reliable experimental work to show that the occipital lobe also sends fibres to the pontine nuclei, despite the opposition to such a view expressed in the work of Dejerine (1893, 1901), Winkleman & Eckel (1929), and Marie & Guillain (1903). Mettler (1935) has demonstrated that in macaque monkeys cortico-pontine fibres arise from the upper lip of the calcarine fissure as well as from the occipito-temporal and occipito-parietal gyri. Kreuser (1891), Flechsig (1903), Bechterew (1900), Meyer (1907), Edinger (1900), Probst (1902), Poliak (1927), and Hösel (1902) have also described an occipital element in this system.

An analysis of these conflicting views makes it apparent that the confusion is, in general, more apparent than real, for, when the relative merits of the various observations recorded are taken into consideration, there is very good reason to believe that cortico-pontine fibres do arise in temporal, occipital, and parietal areas, and that no particular area can be regarded as solely responsible for the origin of this system. Any statements to the contrary are usually based on information derived from a study of pathological material, such as those of Marie & Guillain, Rhein, Mills & Spiller, and Löwenstein, and the doubtful value of such information has already been noted. The evidence afforded by this series of experiments leaves no doubt that cortico-pontine fibres arise in certain parietal, occipital, and temporal fields, and that it is not unlikely that additional fields in these lobes may also contribute to this system. The fact that in these cases the lesions extended, with the exception only of Exp. 6, over more than one cyto-architectural field makes it impossible to determine

the precise fields contributing to this system; where more than one field was included, then any combination of the areas may have given origin to the fibres. However, it seems that the contribution from the parietal and temporal areas exceeds that from the occipital which appears to come mostly from areas 18 or 19, or both. These results, then, not only confirm previous work, but also assist in correlating earlier findings, which, by their isolation, appear to disagree.

The site at which cortico-pontine fibres terminate in the pons is still unsettled. Winkler (1927) carried the parieto-temporo-pontine tracts of his studies into the middle and distal thirds of the pons, with the parietal fibres terminating more distally than the temporal. According to him, the parieto-pontine tract terminates about the medial, ventral, and intrapeduncular part of the peduncular nucleus and, in addition, about parts of the dorso-lateral and ventral nuclei, while the temporo-pontine comes into synaptic relationship with the lateral parts of the ventral and peduncular nuclei, and the lateral and dorso-lateral nuclei. This view for a distal termination in the pons is also shared by Borowiecki (1911), Masuda (1914), and Abbie (1934). Bechterew (1900), on the other hand, believed that the temporo-occipito-pontine tract described by him terminates in the dorsal and dorso-lateral nuclei of the rostral half of the pons. The observations of Spitzer & Karplus (1907), Dejerine (1893, 1901), Thomas & Dumpt (1914), and Marie & Guillain (1903) likewise favour a termination in the rostral part of the pons. In the present study, this system commenced to break up in the rostral third of the pons, and was distributed over the middle and distal thirds of the rostral three-fourths. Any fibres reaching the extreme distal portion of the pons could be traced caudally into the medulla. However, the limitations of this particular method of study make it difficult to name any specific nuclear groups (even if such could be topographically defined with any accuracy) as the terminal focus of the various elements—temporal, occipital, and parietal—of this system, and the only conclusion that can be safely drawn is that the fibres are distributed over the rostral three-fourths of the pons, chiefly in its distal portion, and that they terminate about the lateral and ventral parts of the main nuclear mass, with the parietal fibres terminating more distally than the remainder.

III. THE PONTO-CEREBELLAR SYSTEM

The observations were based on five experiments. The lesions were produced by removing small localized areas of the cerebellar cortex with a diathermy. Here devascularization is not a suitable method, because of the arrangement of the cerebellar surface into folia, which makes it impossible to encircle a given area completely and deprive it of its blood supply.

Anaesthesia was induced as before. To approach the upper surface of the cerebellum an osteoplastic flap was turned down in the occipital region, the occipital lobe gently elevated and the tentorium incised. The lesions on the posterior and inferior surfaces were produced by exposing the cerebellum

through a mid-line suboccipito-cervical incision, with removal of the laminae of the first and second cervical vertebrae. Bleeding was carefully controlled, the deep structures brought together with interrupted linen sutures, and the skin wound closed with a continuous subcuticular stitch. The post-operative treatment was the same as in the previous experiments and all the wounds healed well.

The survival period was approximately six weeks to allow retrograde degeneration in the pontine nucleus to be sufficiently marked so as to be clearly recognizable. The brain was removed, and the lesions examined, measured, and drawn. The pons was separated and divided into suitably sized blocks. These were then treated in the same way as the material used for investigating the structure of the pontine nucleus.

It is not intended in this paper to discuss the relative merits of the various nomenclatures which have been recommended for the subdivision of the cerebellum. The tendency now is to revert to simple classifications, and in this paper that advocated by Ingvar will be used. He subdivided the cerebellum into three lobes, anterior, middle, and posterior, by means of the fissura prima and fissura prepyramidalis. The middle is regarded morphologically as the neocerebellum, and it is believed that this part is especially concerned with the ponto-cerebellar system. The anterior and posterior lobes together make up the palaeocerebellum.

The experiments

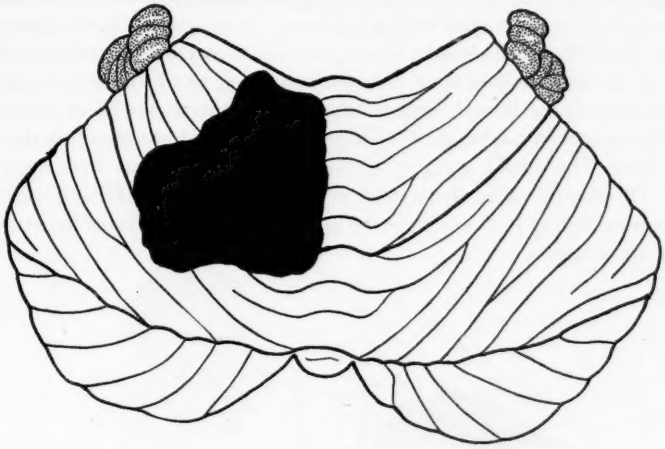
Exp. 7. Monkey 43A. Operation 21 June 1938. Killed 25 July 1938. The lesion was an extensive one on the upper surface of the left anterior lobe. It was irregularly oval in shape with the main diameters measuring 15 and 10 mm. Medially, the border of the lesion involved the left half of the vermis, while posteriorly it extended over the fissura prima to include a small portion of the lobus medius (see Text-fig. 8).

Histological examination showed no apparent change in the left half of the pons. On the right there was a very questionable zone of atrophy in the dorsal nuclear band superiorly, and in one section at the lateral angle of the nucleus mid-way down the pons, but these changes were so doubtful that the series was considered to show no evident change attributable to retrograde degeneration.

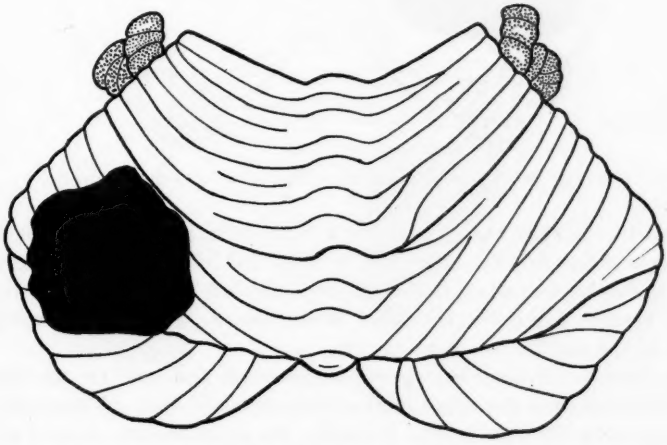
Exp. 8. Monkey 44A. Operation 23 March 1938. Killed 23 May 1938. The lesion was oval in shape, the transverse diameter measuring 10 mm. and the sagittal 6 mm. It was situated on the superior surface of the left lobus medius, between the vermis and the lateral border of the cerebellum, and did not involve the former. The anterior margin of the lesion was in contact with the fissura prima, while posteriorly the lesion crossed the fissura horizontalis for a millimetre or so (see Text-fig. 9).

No histological change was visible on the left side. On the right, degenerative changes, in the form of a diminution of cells with a moderate degree of reactionary gliosis, were observed over approximately the rostral third of the

nucleus. This degeneration was not sharply circumscribed, for many normal cells remained in the areas involved to obscure the degree and extent of the atrophy. Irregular patches showing these diffuse changes were present along the dorsal nuclear band above, where the gliosis was pronounced, and in the



Text-fig. 8. The cerebellar lesion in Exp. 7 viewed from above.

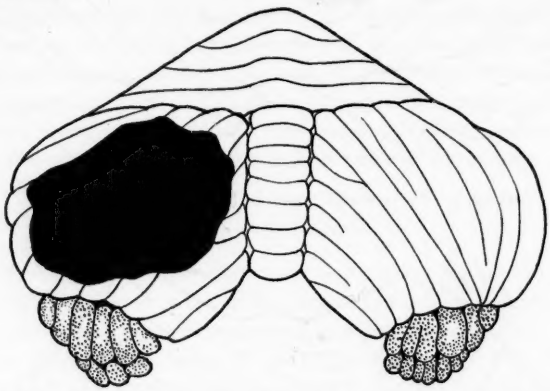


Text-fig. 9. The cerebellar lesion in Exp. 8 viewed from above.

lateral, ventro-lateral, and ventral aspects of the nucleus distally, while there was some thinning of the associated intrapeduncular strands. These changes, though never marked, were most prominent along the dorsal nuclear band and over the lateral aspect of the nucleus.

Exp. 9. Monkey 48A. Operation 30 March 1938. Killed 30 May 1938. The lesion was situated on the postero-inferior surface of the left lobus medius. It measured 13 mm. transversely and 7 mm. sagittally. Medially the lesion almost reached the vermis (see Text-fig. 10).

Degenerative changes were confined to that portion of the nucleus situated in approximately the rostral half of the pons. On the right these changes took the form of a diffuse cell atrophy which produced a marked thinning out of cells, chiefly in the dorso-lateral spur, but also involving, to a lesser degree, irregular areas in parts of the lateral, ventro-lateral, and ventral aspects of the nucleus. A mild gliosis accompanied these changes and was most prominent in the dorso-lateral spur. The dorsal and medial portions of the nucleus were apparently intact. On the left side there appeared to be some doubtful cell-atrophy in areas corresponding to those involved on the right, but this was indistinct and so difficult to assess.

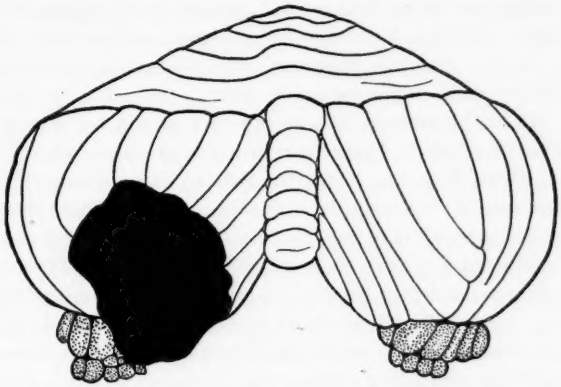


Text-fig. 10. The cerebellar lesion in Exp. 9. Postero-inferior aspect.

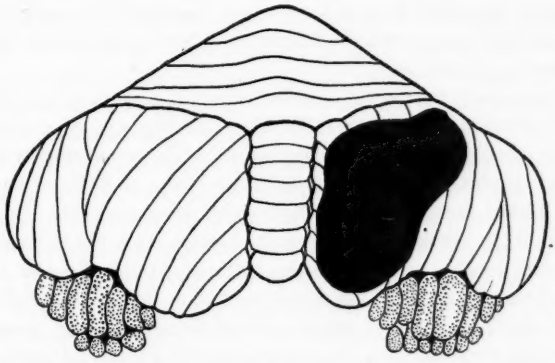
Exp. 10. Monkey 54A. Operation 8 June 1938. Killed 19 July 1938. The lesion was situated on the lower surface of the left lobus medius, and extended round to involve a small portion of the paraflocculus. There was no involvement of the vermis. The lesion measured 10 mm. in the transverse diameter and 5 mm. in the sagittal (see Text-fig. 11).

Degenerative changes were most pronounced in this case. On the right side they extended from the upper limit of the nucleus in the mid-brain above, to half-way down the pons below. Rostrally, the entire nucleus showed atrophic changes which were most marked over the medial three-fourths of the nuclear band. Normal cells, however, were present scattered along the path of degeneration which was well-outlined by a dense line of gliosis. Distally, the changes, though always more marked over the medial aspect of the nucleus, extended to involve the adjacent ventral portion of the nucleus so that the serrations in this region were atrophied, while, in addition, a marked thinning out of cells

was present in the remainder of the nucleus with no obvious gliosis. There was no doubt that the entire nucleus was involved, with the main changes being disposed medially (see Pl. I, fig. 1*b*). Approximately half-way down the pons there was a gradual return to the normal condition, with atrophic changes persisting last of all in the medial portion of the dorsal element of the nucleus. Corresponding areas on the left side were involved to a lesser degree in that the



Text-fig. 11. The cerebellar lesion in Exp. 10. Postero-inferior aspect.



Text-fig. 12. The cerebellar lesion in Exp. 11. Postero-inferior aspect.

cells were not so thinned out, while the marked gliosis observed on the right was absent.

Exp. 11. Monkey 56A. Operation 20 December 1938. Killed 27 January 1939. The lesion was an extensive one and was directed obliquely across the postero-inferior surface of the right lobus medius, just lateral to the vermis. The main diameters measured 14 and 6 mm. (see Text-fig. 12).

The rostral half of the left nucleus showed a patchy cell atrophy, not easily recognized, in its dorsal, lateral, ventro-lateral, and ventral segments, while the associated intrapeduncular strands were also involved. Once again the changes were subtotal, and there was little glial response to accentuate the appearance of atrophy. The changes dorsally were not extensive and were limited to the most rostral four sections of the series. There was some suggestion of a thinning out of cells in the ipsilateral dorsal nuclear band, but this change was ill-defined and was certainly not as marked as that present on the contralateral side.

Before these histological findings are analysed, certain difficulties relating to the identification and assessment of degeneration in this nucleus will be discussed. Retrograde degeneration is usually revealed by an absence or decrease of cells in the presence of a reactionary gliosis, but a pronounced and easily identifiable gliosis is likely to result only in areas where the cells are densely grouped together, and where the atrophy is a circumscribed and total, or almost total one, as, for example, in thalamic nuclei. Where the cells are few in number and scattered, it is unlikely that their atrophy will provoke a glial response of sufficient intensity to be readily recognizable. Thus in the intrapeduncular strands, and over certain other parts of the pontine nucleus where the nerve cells are not densely grouped together but are openly scattered in a linear fashion, this is likely to occur, and since this particular area is also rich in glial cells for the scaffolding of the reticular system which is such a characteristic cyto-architectural feature of the nucleus, it becomes exceedingly difficult to identify and assess subtotal changes in this region in terms of the gliosis present. Again, the possible presence of asymmetrical bilateral changes of the indefinite type already described adds to the difficulty because it prevents one side being used as a control.

It must be stressed that in this series the presence and extent of the atrophy were exceedingly difficult to detect. The degeneration was never circumscribed but was diffuse in character, with many normal cells remaining in the involved areas. Such a persistence of normal cells has three possible explanations. It may be due to the fact that when the lesions were made not all parts of the folia included in the lesion were destroyed. In some only the tips were damaged so that only the pontine cells associated with those parts atrophied. However, it seems doubtful that this alone could be responsible for the diffuse changes present and the number of normal cells remaining in the involved areas. Another possible explanation is that there may be a bilateral representation in the pontine nucleus of the type described by Sinnige (1938) and others. In his experiments, however, he used dogs, and the lesions were mass removals, that is, bilateral changes followed removal of one-half of the cerebellum with involvement of the vermis, while complete removal resulted in total cell atrophy. Ipsilateral changes appeared to be present in these sections but they were indefinite, and certainly much less in degree and extent than those on the contralateral side. It is also likely that the absence of recognizable change in the caudal part of the nucleus is due to the fact that this part has been shown by

Mingazinni (1895), Sinnige (1938) and others to have important bilateral connexions and also connexions with the vermis, so that any changes present might perhaps be obscured by the normal cells persisting. Still another explanation is that there may be no precise localization of cerebellar areas in the pontine nucleus, the latter projecting diffusely over the cerebellum. This probably best accounts for the presence of subtotal changes over relatively extensive and separate parts of the nucleus, the first two factors discussed merely influencing the number of normal cells remaining to obscure the true extent of the atrophy. Bearing these difficulties in mind it will be appreciated that this particular section of the investigation can only provide conclusions of a general nature.

The findings may be summarized as follows:

(1) All the lesions except one involved the lobus medius; in the one exception the lesion was chiefly in the territory of the anterior lobe. Taken collectively, the lesions extended over the superior, posterior, and inferior surfaces of the lobus medius and overlapped.

(2) The atrophy appeared to be almost entirely contralateral in all cases, and involved only that portion of the pons which has been described as the pontine nucleus in a preceding section. Ipsilateral changes could not be definitely excluded. In some cases they were observed, in others they appeared to be absent. When present they were indefinite and of a very minor character compared with those on the contralateral side.

(3) The lesion involving the anterior lobe was not followed by any recognizable retrograde degeneration, suggesting that if this portion of the cerebellum has connexions with the pontine nucleus they cannot be detected by this method. It might be expected that that portion of the lesion extending into the lobus medius would result in some atrophy, but apparently the area so involved was not extensive enough to produce an atrophy of sufficient intensity and detail to be recognized.

(4) The type of degeneration observed, namely the presence of subtotal changes over extensive areas of the nucleus as opposed to any circumscribed patches of atrophy, favours the view that the rostral half of the pontine nucleus projects diffusely over the contralateral lobus medius of the cerebellum. In this way any one part of the cerebellar cortex is, in general, brought into relationship with extensive areas of the pontine nucleus, and vice versa, and this arrangement makes it likely that all parts of the lobus medius are of equivalent value anatomically and physiologically. The absence of any separation of the pontine nucleus into specific and separate nuclear groups is also against the view that there is any functional or spatial localization in this region, though a selective increase in size of certain parts of the nucleus in certain regions is highly suggestive that such an arrangement might be dependent, to some extent, on fibre connexions, cortical or cerebellar. For example, the ventral nucleus is well developed distally, while the dorsal nucleus is most prominent rostrally. Later it will be shown that this feature is probably dependent on the association of the nucleus with the cerebral cortex. The degeneration in Exp. 10

extended to a higher level in the pons than in the other experiments. This would suggest that the inferior surface of the lobus medius is connected with the more proximal parts of the rostral half of the nucleus; in other cases, however, the fields of atrophy overlapped to such an extent that it is impossible to assign the connexions of any particular part of the lobus medius to any particular part of the nucleus in a rostro-caudal plane. The only localization supported by this study is of a general nature, that is, that at least the rostral half of the pontine nucleus projects to the contralateral lobus medius of the cerebellum.

DISCUSSION

There is still lack of agreement as to whether the ponto-cerebellar tracts are crossed, uncrossed, or both. Lewandowsky (1904), Marburg (1922), Sinnige (1938), Mingazinni (1895), and Winkler (1927) have stated that both crossed and uncrossed pathways are present, while the observations of Vejas (1885), Brun (1925), Borowiecki (1911), Masuda (1914), and Uemura (1917) favour a contralateral relationship only. The evidence derived from this study demonstrates that the relationship is a bilateral one, though there is no question that the contralateral contribution predominates. Since only one lesion involved the vermis, and was not localized to it, no comment can be made on the observations of Spitzer & Karplus (1907), Besta (1912), Jakob (1928), Sinnige (1938), and Winkler (1927) who believe that the pontine nucleus is connected with the vermis as well as with other parts of the cerebellum. Concerning the question of the anatomical relationship between the pons and cerebellum, Masuda (1914) has stated that no element of the pontine nucleus projects exclusively to any specific cerebellar area, but that a localization representing principal connexions only is present, in which some areas are connected chiefly with certain parts of the nucleus. Thus he writes that the dorsal nucleus relays chiefly to the lobus quadrangularis, the lateral nuclei to the lobuli semilunares, the medio-ventral to the lobulus biventer, and that in general the caudal part of the pontine nucleus is connected with the anterior part of the cerebellum, and the rostral part of the nucleus with the posterior part of the cerebellum. The connexions described by him indicate that the pontine nucleus projects on to the lobus medius. This view of a relative localization only is shared by Winkler (1927) whose researches suggest that the ventral nucleus projects chiefly to the lateral parts of the cerebellum and lobulus biventer. Abbie's (1934) conclusions, based on purely morphological studies, suggest a localization of a slightly different type. He believes that the pretrigeminal portion of the pons projects to that portion of the cerebellum posterior to the fissura prima and chiefly to what he calls the "declive-tuber complex", while the post-trigeminal part of the pons relays to the anterior cerebellar lobe and chiefly to the culmen in that region. In this series, only one lesion (Exp. 7) involved the anterior lobe, and there no degeneration could be detected in the pons, which would suggest that the pontine nucleus does not project to this lobe of the cerebellum.

The results of this investigation show that the lobus medius, which represents the neocerebellum, is connected with the rostral half of the pontine nucleus, and that retrograde degeneration in the pons following lesions of this lobe is of such a type as to exclude a precise projectional localization of the cerebellum in respect of the nucleus. Thus all parts of the nucleus appear to be diffused over the cortex of the cerebellum.

Relationship between the cerebral and cerebellar cortex. These experiments have demonstrated the manner in which the cerebral cortex projects on to the pons, and the pons on to the cerebellum. Correlating these two groups of findings, it now becomes possible to establish experimentally a more accurate anatomical relationship between these two cortical systems.

The relationship is almost exclusively a contralateral one. The cerebral cortex projects to the rostral three-fourths of the pontine grey, and it has been noted that it is precisely this area which, in turn, projects to the cerebellum. An analysis of the cerebellar lesions indicates that for the most part they are exclusively neocerebellar, so that we now have experimental proof that the cerebral cortex and neocerebellum are linked anatomically, and that, so far as one may conclude from these lesions, the cerebral cortex is connected to no other part of the cerebellum.

The tract degeneration observed in this investigation suggests that the contribution from the temporal, parietal and occipital areas exceeds that from the frontal areas, and that these two major systems project to different parts of the pontine grey, the former chiefly to the ventral and lateral elements in the distal part of the rostral three-fourths of the pontine nucleus, and the latter chiefly to the dorsal nucleus in the rostral portion of the pons, though there is a considerable overlap of fibres in a rostro-caudal plane, some temporal fibres, for example, terminating as far rostrally as the upper border of the pons. So far as the cerebellum is concerned, the cyto-architectural changes in the pontine nucleus suggest that all aspects of approximately the rostral half of that nucleus project diffusely over the cortex of the lobus medius, so that there can be no division of either into specific areas, each with its precise and exclusive fibre connexions. Thus it seems that all the cortical areas studied ultimately project over extensive areas of the lobus medius. Such a relationship suggests that the various parts of that lobe have equivalent values anatomically and physiologically, and this is a point which appears to be borne out by clinical observation. If such be the case, then any elaboration of the pontine nucleus in the form of an increase in size of certain portions of it at certain levels might be expected to be dependent on cortical relationships rather than on cerebellar connexions, and any progressive differentiation of the nucleus would be more dependent on changes in the former than in the latter.

SUMMARY

1. The topography of the pontine nucleus in the macaque monkey is described.

2. The results of an experimental investigation on the projection of the cerebral cortex on the pons, and the pons on the cerebellum in the macaque monkey are recorded and discussed.

3. From the observations made it is concluded that:

(i) Cortico-pontine fibres take origin in the frontal, parietal, occipital and temporal lobes of the brain and terminate in the rostral three-fourths of the ipsilateral pontine nucleus.

(ii) The fronto-pontine tract receives fibres from area 6, but probably none from areas 8 and 9. These were the only fields of the frontal lobe which were investigated. The tract occupies the medial segment of the cerebral peduncle and terminates chiefly about the dorsal and medial portions of the nucleus in the rostral half of the pons.

(iii) The parietal, occipital and temporal fibres are associated in a common pathway which occupies the lateral segment of the cerebral peduncle and terminates chiefly about the ventral and lateral aspects of the rostral three-fourths of the pontine nucleus, the parietal fibres terminating more distally than the remainder. The cortical fields giving origin to these fibres and the relative contributions from those fields are discussed.

(iv) The rostral half of the pontine nucleus projects chiefly on to the contralateral lobus medius of the cerebellum. There is no strict projectional localization in the sense that a particular part of the nucleus projects exclusively to any specific cerebellar area. All aspects of the nucleus appear to project diffusely over the surface of the lobus medius.

(v) Correlating these findings it may be assumed that the cerebral cortex projects to the contralateral lobus medius of the cerebellum, which represents the neocerebellum, in such a way that the various parts of that lobe have equivalent values anatomically and physiologically, and that any differentiation in the nucleus is more dependent on its cortical relationships than on its cerebellar connexions.

ACKNOWLEDGEMENTS

I am deeply indebted to Prof. Le Gros Clark at whose suggestion this investigation was undertaken and in whose laboratory it was carried out. His generous advice and kindly direction are favours which I much appreciate. Three of the cerebellar lesions were performed by him, and a fourth by Dr Schlesinger, and these I wish to acknowledge. I should also like to express my thanks to Mr E. Thompson for the preparation of the sections used in this study, and to Mr W. Chesterman for the photographs which accompany this paper.

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EXPLANATION OF PLATE I

Fig. 1. A micro-photograph, from Exp. 10, of a transverse section of the pontine nucleus at a rostral level in the pons, which illustrates the type of retrograde degeneration observed in the nucleus. ×30. *a* is the ipsilateral nucleus, and *b* the contralateral. The lateral aspect of the nucleus is to the right in *a* and to the left in *b*, while the ventral aspect in both cases is directed downwards. The three characteristic features of the atrophy are well shown: (i) the changes are bilateral, those in the contralateral nucleus being pronounced, while the ipsilateral nucleus shows only a minor involvement; (ii) the changes are general, all aspects of the nucleus being involved; (iii) the changes are subtotal.

Fig. 2. Micro-photograph to illustrate the type of degeneration revealed by the Marchi method. ×55. The section is from Exp. 6, the temporal lesion, and the site is at the junction of the mid-brain and pons in the lateral third of the peduncle.

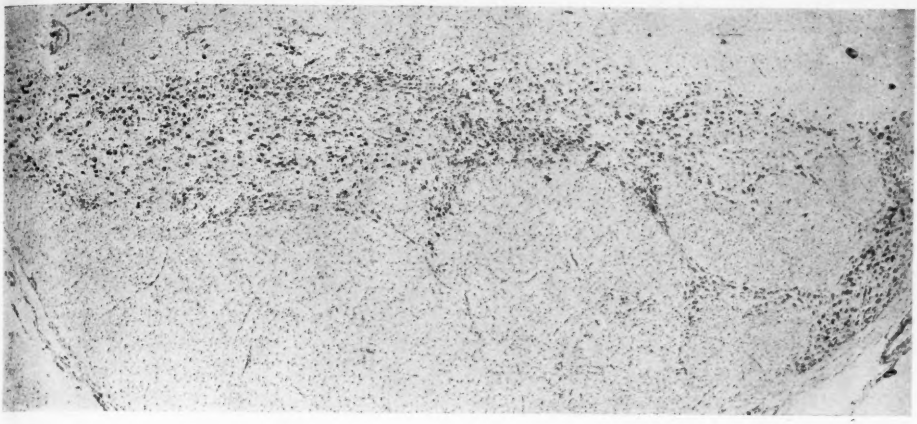

Fig. 1*a*.

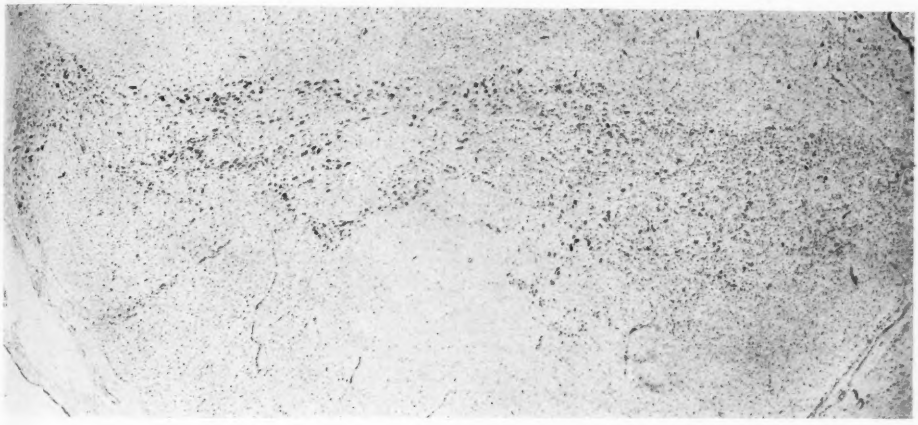

Fig. 1*b*.

Fig. 2.

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ON THE RELATIVE POSITION OF THE HIPPOCAMPUS AND THE CORPUS CALLOSUM IN PLACENTAL MAMMALS

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For many years what may be called the classical theory of the origin of the corpus callosum has received general acceptance from anatomists in all countries. It has come to form part of a background of morphological knowledge against which more detailed investigations are viewed, and it was perhaps the most important single contribution made by Elliot Smith to anatomical science. For these reasons the appearance of a new theory (Abbie, 1939) is an important event. This new theory, if true, upsets the main homologies which appeared to have been so firmly established by Elliot Smith.

The main features of Elliot Smith's theory are too well known to need recapitulation. Abbie's theory differs fundamentally since it demands that callosal fibres should grow through subicular cortex above the hippocampus, instead of through the dorsal limb of a bilaminar hippocampal commissure like that of marsupials, as Elliot Smith supposed. The difference is clear cut; according to Abbie the corpus callosum must always lie dorsal to the fascia dentata and cornu ammonis or their vestiges. In Elliot Smith's view this relationship must be reversed. Many of the observations reported by Abbie are obviously incompatible with the classical theory, and some throw doubt on the accuracy of Elliot Smith's original descriptions. For these reasons a reinvestigation of the problem seemed desirable.

The main observations on which Abbie's statement, that the corpus callosum is developed dorsal to the hippocampal formation, is based, are as follows; with unimportant exceptions they have all been made in each of a representative series of mammals. In the first place, according to Abbie, the pre-commissural hippocampal rudiment lies below the genu of the corpus callosum, and only subicular cortex extends above it into continuity with the indusium. Secondly, around the splenium the indusium is continuous again with subicular cortex and not with fascia dentata or cornu ammonis. Thirdly, the fascia dentata and the cornu ammonis extend forwards under the corpus callosum, accompanied by a variable amount of subicular cortex, to end in or just behind the hippocampal commissure. There should be no hippocampal flexure such as Elliot Smith described. Lastly, the hippocampal commissure or psalterium consists of two distinct parts, dorsal and ventral. Such a division of the psalterium has been recognized before (Kölliker, 1894; Elliot Smith, 1898; and others), but in Abbie's theory the dorsal part of the psalterium (the fornix dorsalis of his figures) is a new commissure peculiar to

placental mammals, and it crosses between the subicular and not the fimbrial margins of the hippocampal formation.

All these observations are inconsistent with Elliot Smith's theory, and for the most part contradict his findings. The present work will be limited to a reinvestigation of the brain in four mammals by ordinary neuro-histological methods with reference to these observations. The mammals used are a bat (*Eptesicus pumilus*), the rat, mouse and cat. The bat, *E. pumilus*, belongs to a subfamily of the Vespertilionidae; the two species investigated by Elliot Smith belonged to other subfamilies of the same family.

MATERIAL

This work is based principally on the following series of sections:

No.	Animal	Plane of section		Thickness μ	
1	Bat (*E. pumilus*, adult)	Transverse	Celloidin	25	Toluidin blue
2	Cat (adult)	Transverse	Paraffin	10	Toluidin blue
3*	Rat (adult)	Transverse	Paraffin	10	Davenport silver
4*	Rat (adult)	Sagittal	Paraffin	10	Davenport silver
5	Rat (adult)	Sagittal	Paraffin	10	Toluidin blue
6	Rat (adult)	Horizontal	Celloidin	25	Toluidin blue
7	Mouse (half-grown)	Transverse	Paraffin	15	Toluidin blue
8	Mouse (half-grown)	Horizontal	Paraffin	10	Toluidin blue

* In series 3 every tenth, and in series 4 every sixth section was mounted separately and stained with toluidin blue.

For the limited purpose of this work it was found that the half-grown mouse did not differ significantly from the adult rat. The sections obtained from the mouse were particularly suitable for photography, and have therefore been used in preference to the rat sections for some of the illustrations.

A number of series of late rat embryos, of newborn rat brains, and one of the brain of a rat 10 days old were available. They were stained by various silver impregnation methods, toluidin blue or haematoxylin and van Gieson's stain. It is unnecessary to report any of these series; they were useful in corroborating the results obtained from the adult brains.

THE BAT (*EPTESICUS PUMILUS*)

The conditions in this brain are so clear and uncomplicated that the illustrations almost explain themselves.

The hippocampus arches dorsally and medially in the usual way, and comes to occupy a large part of the medial surface of the hemisphere behind the commissures (Pl. I, fig. 1). The ordinary characteristics of fascia dentata and cornu ammonis are shown (so far as these can be revealed by toluidin blue material) and there is a short subicular zone of transition to the neocortex. The fimbria is a massive structure which forms a large part of the medial wall

of the lateral ventricle. Anteriorly the curvature of the cornu ammonis becomes flattened, and the lower limb of the fascia dentata separates from the upper (Pl. I, fig. 2, left side), and soon disappears (Pl. I, fig. 2, right side, and fig. 3). At the same level the fibres of the fimbria turn medially, and a large vein, lying in the depression between fimbria and fascia dentata, turns in the same direction to enter the great longitudinal fissure (Pl. I, figs. 2, 3). The "splenium of the corpus callosum" next appears (Pl. I, fig. 4) crossing the mid-line. It consists largely of fibres from the fimbria, but owing to the flattening of the cornu ammonis the way is clear for the entry of fibres from the neocortex as well. It is obvious in this and in the following sections that the flattened cornu ammonis forms the thin layer of nerve cells, the indusium, on the upper surface of the splenium. For a short distance the fascia dentata is recognizable along its medial margin (Pl. I, fig. 4, left side). In fig. 5 the massive hippocampal commissure or psalterium appears in its full extent. Dorsally it contains neocortical fibres (the corpus callosum) above which is found the indusium or hippocampal vestige. Anteriorly the psalterium gives place to the septal nuclei (paraterminal body) limited dorsally by a small corpus callosum (Pl. I, fig. 6). The indusium is present as before, and, except for differences in the proportionate development of the various structures, this appearance can be seen in a transverse section through the same region in any placental mammal. Anterior to the genu the indusium can be followed into the ventro-medial margin of the cortex immediately above the septal or paraterminal nuclei (Pl. I, fig. 7). There is no cortical formation of any kind ventral to the corpus callosum.

It is perfectly clear that the conditions described here for *Eptesicus* are almost identical with those found by Elliot Smith (1897*b*, 1898) in *Nyctophilus* and *Miniopterus*. The hippocampal formation passes entirely dorsal to both the psalterium and the corpus callosum. Above the latter it is represented by the indusium, in the most posterior parts of which vestiges of the cornu ammonis and fascia dentata can be recognized. The indusium (hippocampus) extends forwards beyond the genu where it is represented, as Elliot Smith said (1897*b*), by a few small cells at the junction of the precommissural area and the general cortex.

Although from toluidin blue material no precise description of fibre tracts can be given, it is also clear that the general morphology of the commissures agrees very closely with Elliot Smith's description. The psalterium is a thick lamina of fibres, set almost vertically; it joins a second thinner lamina above at an acute angle which points dorsally and caudally. This second lamina slopes forwards and ventrally, above the paraterminal or septal region. It is the corpus callosum, and its posterior border where it joins the psalterium is the splenium. The resemblance between the corpus callosum and psalterium on the one hand, and the two laminae of the marsupial hippocampal commissure on the other, is so close, that it is impossible to avoid Elliot Smith's conclusion that they are comparable structures.

THE RAT AND MOUSE

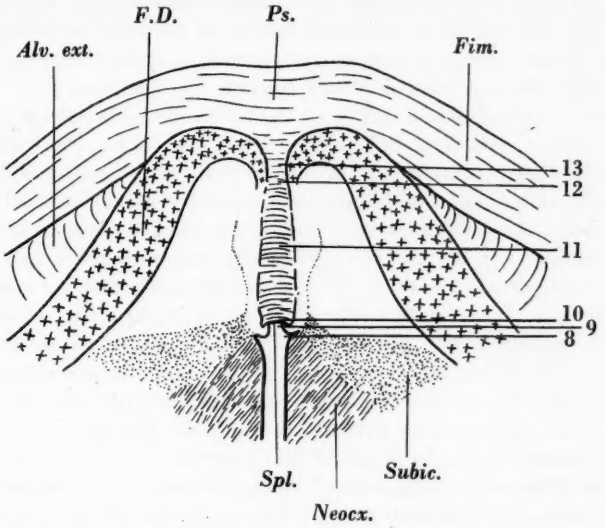
The precommissural hippocampal rudiment is rather well developed, and is represented by a definite compact layer of cells ventral to the neocortex and anterior to the genu. At its dorsal border there is a narrow zone of transition to the neocortex. In this zone cells are rather scanty and it probably represents the subiculum. In these series the genu of the corpus callosum makes its appearance not in the subicular zone, but in the hippocampal rudiment below it, nearer to its upper than its lower margin. It divides the rudiment into two parts; one, small, which persists above the genu as the indusium, and the other, more extensive dorso-ventrally, which persists for a very short distance below the genu. There is no obvious difference in structure between the parts which pass above and below the genu.

No accurate estimate of the extent to which the hippocampal rudiment passes below the genu can be made from a transverse series. The apparent extent will vary according to the plane of section. The corpus callosum in the rat and the mouse slopes downwards and forwards (Text-fig. 2), so that sections cut in the ordinary way, perpendicular to the long axis of the brain as a whole, will cut across the corpus callosum obliquely. In following through such a series the first part of the corpus callosum to appear will be the upper surface of the genu and not its true anterior extremity. The series will therefore give an exaggerated idea of the extent to which the hippocampal rudiment is found beneath the genu. The true condition of affairs is shown without ambiguity in sagittal sections. Pl. III, fig. 14, from an adult rat brain, makes it clear that the hippocampal rudiment extends only very slightly beneath the genu, and predominantly above it to become the indusium. This section was chosen for illustration because it shows the maximum extent of the subgenual hippocampus in this particular series.

Towards the posterior end of the corpus callosum the indusium becomes extremely thin. It can be followed round the posterior surface of the splenium quite easily in transverse and sagittal sections (less easily in horizontal). It lies close to the median plane, and remains quite distinct from the subiculum which is more laterally situated. It joins the dorsal infra-callosal margin of the hippocampus below the splenium. These features are shown for the mouse in Pl. II, figs. 9, 10.

Subicular cortex is, of course, easily recognizable behind the splenium (Pl. II, fig. 8). It consists of a broad layer of cells, fairly widely spaced, and irregularly pyramidal in shape. No lamination can be seen in it. It is quite distinct from the very definite layer of cells in the cornu ammonis, but appears to blend with the deeper layers of the neocortex. Preserving these characteristics, the subiculum passes dorsally round the lateral part of the splenium; on the upper surface of the corpus callosum it is only doubtfully recognizable between the indusium and the neocortex. Wherever it can be recognized, it is always quite as distinct from the indusium, which lies nearer the median plane, as it is in other situations from the cells of the cornu ammonis.

The subiculum, as described above, also extends under the splenium (Pl. II, fig. 10) where it still lies lateral to the indusium which has followed it round. It can be recognized, however, only for a very short distance, and ends along a horizontal line, nearly transverse, beneath the corpus callosum (Text-fig. 1). Its place is taken by a compact layer of cells which has in general the characteristics of the cornu ammonis. This interpretation is illustrated in Text-fig. 1, a graphical reconstruction of the structures on the under surface of the corpus callosum in the mouse. The usual figures, in which these structures are represented as projected on the median sagittal plane, are



Text-fig. 1. A diagram (based on a graphical reconstruction) of the structures on the ventral surface of the corpus callosum in the mouse. Magnification ×30. The region is seen from the ventral aspect. The right and left hippocampal formations almost meet in the mid-line anteriorly; in the diagram they have been separated slightly to show the fibres of the psalterium between them. The lines numbered 8–13 indicate the levels of the sections illustrated in Pl. II.

unsatisfactory, since the hippocampal formation approaches the median plane obliquely in a flat curve over the dorsal surface of the midbrain and thalamus. It is far more accurately represented from a ventral than from a medial aspect.

The conditions at the extreme anterior end of the hippocampus are more difficult to interpret, particularly in transverse sections. The fascia dentata approaches the mid-line obliquely. It disappears from the series just behind the ventral part of the psalterium, but the appearance that the last traces present varies considerably, being influenced by small differences in the plane of section. Usually the two laminae become separate and the upper disappears before the lower inside a circle of cells of the cornu ammonis. There is no need to describe these appearances in detail; it is almost impossible to interpret

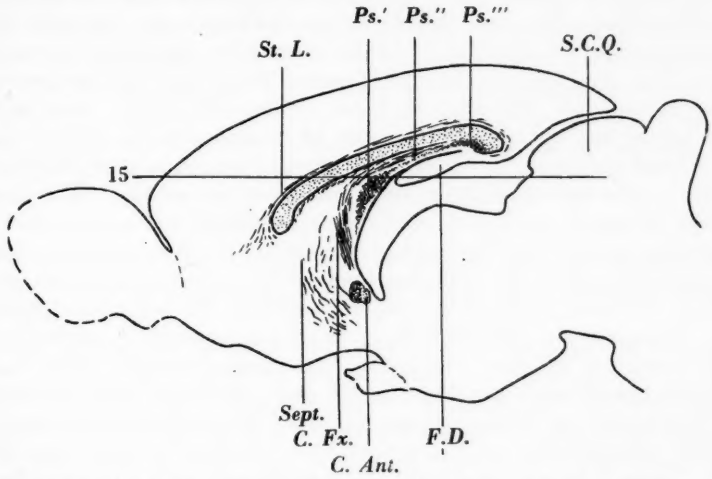
them completely without making a reconstruction in three dimensions. One feature, however, has been observed in all series, and its meaning is fairly clear. From the fascia dentata, just before it disappears from the series, a tail of cells passes dorso-medially to come into intimate contact with the infra-callosal margin of the cornu ammonis (Pl. II, fig. 13), and can be traced for a short distance along that margin towards the splenium (Pl. II, fig. 12). There is in fact some evidence at least for the presence of a flexure of the fascia dentata which carries backwards a small tail of the characteristic granule cells very close to the mid-line. Evidence that is easier to interpret and which leads to the same conclusion can be obtained from sagittal and horizontal sections. Pl. III, fig. 15, a horizontal section of an adult rat's brain, shows the fascia dentata cut twice; laterally, where it is approaching the mid-line, and medially, where it is turning back towards the splenium.

The dorsal infra-callosal border of the cornu ammonis, along which this recurrent tail of cells from the fascia dentata runs for a short distance, is identified by Abbie as subicular. As can be seen in the photographs (Pl. II, figs. 11, 12), it has none of the obvious characteristics of subicular cortex. At some levels its cells are rather less closely packed than in most of the cornus ammonis (e.g. fig. 11), but throughout, and with reference only to its intrinsic characteristics, it would be identified naturally with the cortex of the cornu ammonis. It shows two other features of importance. In most of its course it is separated from the main cell lamina of the cornu ammonis by an indentation (Pl. II, fig. 11), and close to the splenium, by obvious subiculum (Pl. II, fig. 10). These observations, particularly if they are taken in conjunction with what has been said about the fascia dentata, lend themselves readily to interpretation in terms of Elliot Smith's theory, i.e. as the result of a flexure of the whole hippocampal formation beneath the corpus callosum. As can be seen in the reconstruction (Text-fig. 1), the flexure is acute, but it affects the subicular border of the hippocampus only slightly; the recurved part of the fascia dentata is vestigial. For these reasons the reduplication of the whole hippocampal formation which one might expect to see in transverse sections does not appear.

It is the writer's opinion that, after a careful examination of transverse, sagittal and horizontal sections, this interpretation is the only one possible. The limited amount of evidence that it is possible to reproduce in a short paper is less convincing, but it is at least enough to show that the infra-callosal border of the cornu ammonis is not typically subicular, and that it does present certain features which are not explained by Abbie's theory, even if they do not absolutely exclude it.

There remains the question of the hippocampal commissure or psalterium and its division into dorsal and ventral parts. A sagittal section, stained for fibres, shows that this subdivision is not precise (Text-fig. 2). There is a fairly bulky mass of transverse fibres beneath the splenium (or rather forming a part of the splenium), *Ps.'''*, and another, much further forward and more ventrally

situated below the hippocampal formation *Ps.'*. The latter is obviously derived from the fimbria. Between the two is a thin lamina of decussating fibres, most of which cross obliquely, *Ps.''*, but there is no point at which it can be said that the dorsal psalterium ends and the ventral begins. Nor does Abbie give any criterion by which these two subdivisions can be distinguished precisely. Further laterally the hippocampus extends anterior to the fascia dentata shown in Text-fig. 2 so that in some transverse sections commissural fibres may be seen both above and below the anterior end of the hippocampal formation. If the series is followed forwards these two sets of fibres must come into apposition as Abbie describes and as is shown in his figures (of *Erinaceus* and *Cynopterus*). The point or line of apposition is marked by nothing except



Text-fig. 2. Drawing of a parasagittal section of an adult rat's brain stained by the Davenport silver method. The commissures have been drawn as seen in the median plane. The line marked 15 shows the plane of the section illustrated in Pl. III, fig. 15.

the final disappearance of the hippocampal formation. The anterior end of this formation is rounded, and its actual point of disappearance will vary according to the obliquity of the transverse sections. There is nothing either in transverse or in sagittal sections shown by the commissure itself to suggest a precise subdivision into two parts. The dorsal and ventral parts of the psalterium are continuous and there is no sudden transition from one to the other.

It is true, of course, that the fibres that cross in the dorsal part have had a different origin and course from those that cross ventrally. The latter have all run in the fimbria and have come from the more distant parts of the hippocampus. The former have come from nearer parts of the hippocampus and have never entered the fimbria at all. This difference is quite adequately explained by Elliot Smith's theory. According to Elliot Smith the psalterium

of placental mammals is formed from the ventral limb of a bilaminar hippocampal commissure like that of marsupials. It has been stretched out in nearly all placental mammals by the enlargement posteriorly of the dorsal limb into which fibres from the neocortex have grown to form the corpus callosum. Since the ventral limb of the marsupial commissure consists entirely, or almost entirely, of fimbrial fibres, it follows that the formation of the fimbria itself must be affected by this stretching. Some of its fibres become stretched over the upper surface of the hippocampal flexure, and form the dorsal part of the psalterium (*Ps.''* and *Ps.'''*, Text-fig. 2), losing their fimbrial character in the process. There is no complete reversal in the direction taken by some of the alveus fibres in placental mammals, as postulated by Abbie. In the marsupial, where the commissure is undisturbed by the advent of neocortical fibres, fibres from the alveus enter the fimbria very obliquely. They run, not transverse to the long axis of the hippocampus, but along it, gradually approaching and finally joining the fimbria. The stretching that occurs in placental mammals (according to Elliot Smith) will displace many alveus fibres arising from the nearer parts of the hippocampus, which will then cross in a "dorsal psalterium" without ever entering a defined fimbria. The ventral lamina of the marsupial commissure could not be stretched in this way without producing some such effect. It is reasonable to conclude that the appearance of two hippocampal commissures in transverse sections of some placental brains is a natural consequence of the enlargement of the corpus callosum in the way that Elliot Smith described. He was himself familiar with this appearance, and with its cause, as the figures he published in his paper (1897*a*, p. 44) show. The fact also that this appearance of two hippocampal commissures is not found in the *Microchiroptera*, where the corpus callosum is still very small, is further support for Elliot Smith's interpretation.

This particular aspect of the problem is not clarified by Abbie's use of the term "fornix" for both unilateral and commissural fibres. Elliot Smith, in the latest of his main series of papers on this subject (1898), defined the fornix system very precisely. The terms fornix longus, ventralis or dorsalis he reserved for fibres which remained on the same side of the brain as their origin. The term fornix transversus or psalterium he kept for commissural fibres of the hippocampus. This terminology is, of course, arbitrary and is open to criticism in some respects. It may be impossible to separate longitudinal unilateral fibres so definitely from commissural fibres, but until more precise knowledge of the connexions and interrelationships of these systems is obtained, it undoubtedly increases clarity of description to do so. The fornix longus and fornix superior of Abbie's paper appear to be in his view only parts of a single commissure, the anterior fibres of which have been stretched into a longitudinal direction. There is no evidence in the fibre-stained rat series to suggest that any of the fibres found in the position labelled by Abbie "fornix longus" cross the mid-line. A few may do so, but they must be very few indeed, and there is no doubt that the fibres in this system are predominantly homolateral. They

form the greater part of Elliot Smith's fornix dorsalis, and there is no reason why they should be included in the commissures at all. Similar longitudinal fibres, such as the striae Lancisii and the "fibrae perforantes" of the corpus callosum, clearly visible in the sagittal rat series, are ignored by Abbie. Their presence received an adequate explanation in Elliot Smith's papers (see particularly 1896*b*).

The condition in the mouse and the rat can be summarized as follows. The precommissural hippocampal rudiment is directly continuous with the indusium above the genu. It extends comparatively slightly below the genu. Round the splenium the indusium is continuous with a cortical lamina which has the characteristics of cornu ammonis rather than of subicular cortex, and there is considerable evidence that a hippocampal flexure, in Elliot Smith's sense, is present. There may be some doubt about the detailed interpretation of the recurrent limb of this flexure, and the fascia dentata which accompanies it is vestigial. It lies medial to the main part of the hippocampal formation, and not above it as shown in diagrams projected on the median sagittal plane. Finally, the appearance of a dorsal and a ventral psalterium can be interpreted quite naturally in terms of Elliot Smith's theory.

THE CAT

The condition in the cat has been investigated less completely and is reported only because it shows certain features of the hippocampal flexure particularly clearly.

The cat's brain is large enough for macroscopic examination. On the under surface of the corpus callosum, the midbrain and thalamus having been removed, it can be seen very clearly that the fascia dentata, as a macroscopic gyrus, after running forwards on the under surface of the corpus callosum and psalterium, bends first medially and then posteriorly. It can be traced back close to the mid-line, diminishing in size, until it disappears round the splenium on to the dorsal surface of the corpus callosum. The two limbs of this flexure of the fascia dentata are separated by a prominent tubercle the surface of which passes without any interruption into that of the general cortex posterior and lateral to it. This appearance is familiar, of course, to most anatomists, and can be seen in the brains of many placental mammals. It was interpreted by Elliot Smith as a hippocampal flexure, which it certainly appears to be. According to Abbie's theory no such flexure should occur.

Sections cut through this region in the transverse plane show exactly what the macroscopic appearance would lead one to expect. Near the anterior end of the flexure (Pl. III, fig. 16) both cornu ammonis and fascia dentata are cut twice. These are the two limbs of the hippocampal flexure, and each contains the typical hippocampal differentiations. They are separated by a narrow subicular zone. Posteriorly, the medial limb (the upper limb in Elliot Smith's diagrams) diminishes in size. Before it reaches the splenium it loses its differentiation into cornu ammonis and fascia dentata, and is reduced to a

thin indusium which is continuous round the splenium with the indusium on the dorsal surface of the corpus callosum. At the same time the subicular zone between the limbs enlarges to form the prominent tubercle seen macroscopically.

These conditions are so clear and so easily verified, that they need no further discussion. A hippocampal flexure, possessing all the expected characteristics, does exist in the cat's brain.

CONCLUSIONS

The conditions found in the bat and the cat unequivocally support the homologies put forward by Elliot Smith for the commissures and hippocampal vestiges in the placental brain. It is true that the present description, so far as these two animals are concerned, is based on two series of sections only. An examination of further material might add descriptive detail to the original accounts given by Elliot Smith, but it is unlikely that such detail would have much bearing on the general theory. The bat demonstrates extremely clearly the essential similarity between the commissures and the hippocampal formation in placental and marsupial mammals. As Elliot Smith realized, the morphological relation between the corpus callosum and psalterium on the one hand, and the dorsal and ventral limbs of the bilaminar hippocampal commissure of marsupials on the other, is as certain as any morphological relation can be in the absence of direct palaeontological evidence. In the cat the presence of a hippocampal flexure is extremely obvious. It is accounted for satisfactorily by Elliot Smith, and indeed follows naturally from his demonstration of the relationships that exist in more primitive mammals.

The condition in the rat and the mouse is less self-evident. In a brain like that of the bat, where no hippocampal flexure exists (the corpus callosum has not extended posteriorly beyond the psalterium) serial transverse sections give a perfectly clear picture. In the cat, where the flexure is very well developed, so that the two limbs lie parallel for a short distance and in the same plane, transverse sections are again easy to interpret. In the rat and the mouse the flexure is less well developed; the two limbs are not parallel and the two borders of the hippocampus are affected unequally, the fimbrial border and the fascia dentata being bent acutely, but the subicular border only indented slightly beneath the corpus callosum. For these reasons transverse sections give a confusing picture, and need for their interpretation series cut in other planes. Even so, the appearance is not so obvious as in the cat, mainly on account of the poor development of fascia dentata in the recurrent limb of the flexure. When these differences are taken into consideration the findings in the rat and mouse fall into line with those in the bat and cat. It would appear from Abbie's paper that the conditions in the hedgehog and in the larger bats are intermediate between those in the small bats and the rat. They may therefore present similar and perhaps greater difficulties in interpretation from transverse sections.

Evidence has been produced that in one placental mammal at least the hippocampal formation lies entirely dorsal to the corpus callosum; and that in a more advanced placental mammal a very definite hippocampal flexure occurs, suggesting strongly that the infra-callosal position of part of the hippocampal formation in such mammals is secondary. It has been shown that intermediate conditions, found in such mammals as the rat, although more difficult to interpret from serial sections, lead to the same conclusions. These relationships were clearly described by Elliot Smith, and no general theory of the origin of the corpus callosum which does not account for them can claim serious attention. Elliot Smith's early observations, though they may be amplified and perhaps modified in detail, were essentially correct. The theory which he advanced to account for them still stands, and there is no valid reason why it should be abandoned.

In conclusion, the great phylogenetic stability of the vertebrate commissures should be stressed. The fibres which they transmit may vary both in their origin and destination, but the pathways they follow between the hemispheres have remained essentially the same. Not the least advantage of Elliot Smith's theory is that it accounts for the complex arrangement of the commissures in placental mammals without postulating the development of any essentially new pathway between the hemispheres.

SUMMARY

The relationship between the hippocampal formation or its vestiges and the corpus callosum and psalterium has been described from transverse serial sections stained by toluidin blue, in a bat (*Eptesicus pumilus*) and the cat. Similar observations have been made in the rat and mouse, using, in addition, sagittal and horizontal sections and a silver impregnation method as a fibre stain.

These observations have led to the conclusion that the corpus callosum and psalterium of placental mammals lie morphologically ventral to the hippocampus or its vestiges. This relationship is most clearly seen in the bat. In mammals with a larger corpus callosum (e.g. the cat) a hippocampal flexure is formed beneath the hinder part of the corpus callosum as Elliot Smith originally described. In the rat and mouse this flexure is less clearly seen than in the cat.

In general the evidence found supports the theory put forward by Elliot Smith for the origin of the corpus callosum, and the homologies between the placental and marsupial commissures that he established. No evidence has been found for Abbie's view (1939) that the corpus callosum has grown through subicular cortex above the hippocampal formation.

ABBREVIATIONS USED IN THE FIGURES

Alv.Ext.	Extra-ventricular alveus	*Ind.*	Indusium griseum
C.A.	Cornu ammonis	*Neocx.*	Neocortex
C.Ant.	Anterior commissure	*Ps.*	Psalterium
C.C.	Corpus callosum	*S.C.Q.*	Superior corpus quadrigeminum
C.Fx.	Column of fornix	*Sept.*	Septum or paraterminal body
F.D.	Fascia dentata	*Spl.*	Splenium of corpus callosum
F.D.'	Recurrent limb of fascia dentata	*St.L.*	Stria Lancisii
Fim.	Fimbria	*Subic.*	Subiculum

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EXPLANATION OF PLATES I–III

Plate I

Figs. 1–7. Transverse sections of the brain of a bat (*Eptesicus pumilus*), 25 μ thick, stained by toluidin blue. Magnification $\times 27$. The sections are arranged in order in a frontal direction. Fig. 1 is separated from fig. 2 by 75 μ; figs. 2–5 are each 25 μ apart; fig. 6 is 150 μ anterior to fig. 5, and fig. 7 is 75 μ anterior to the genu of the corpus callosum.

Plate II

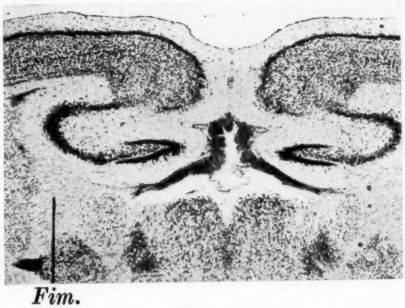
Figs. 8–13. Transverse sections of the brain of a mouse (about half-grown), 15 μ thick, stained by toluidin blue. Magnification $\times 90$. Only structures in the immediate neighbourhood of the corpus callosum are shown. The levels of the different sections are shown in Text-fig. 1.

Plate III

Fig. 14. Parasagittal section through the genu of the corpus callosum and adjacent cortex in an adult rat, 10 μ thick, stained by toluidin blue. Magnification $\times 80$. The section is very close to the median plane and shows the precommissural hippocampal rudiment passing above the genu to become the indusium.

Fig. 15. Horizontal section of adult rat's brain, 25 μ thick, stained by toluidin blue. Magnification $\times 30$. Only structures immediately in front of and behind the ventral part of the psalterium are shown. The plane of section is indicated in Text-fig. 2.

Fig. 16. Transverse section of a cat's brain, 10 μ thick, stained by toluidin blue. The section passes through the anterior part of the hippocampal flexure beneath the corpus callosum.



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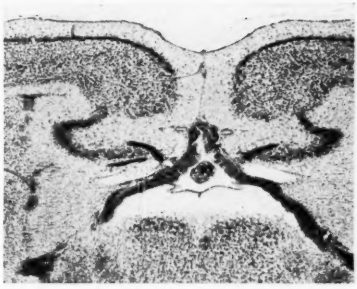

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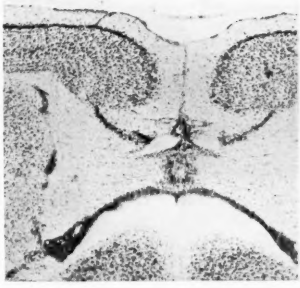
Fig. 3.



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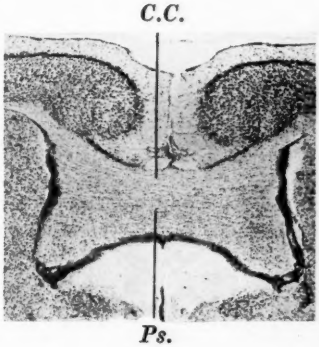

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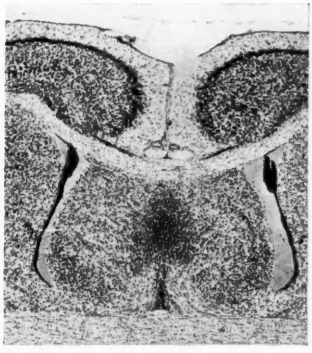

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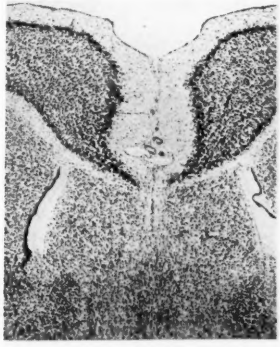

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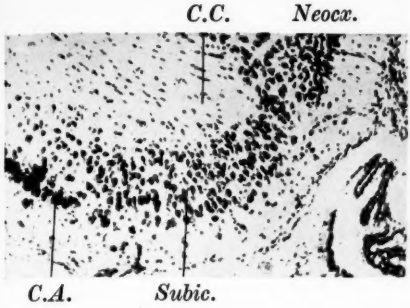

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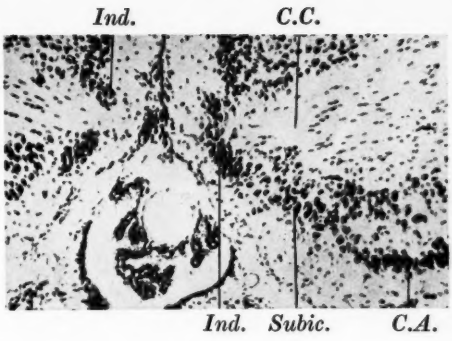

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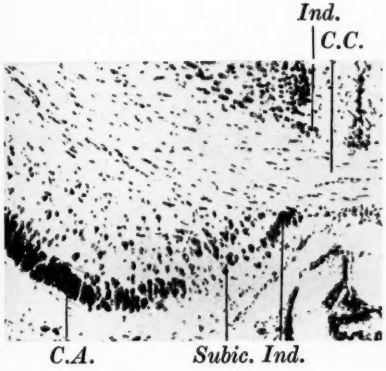

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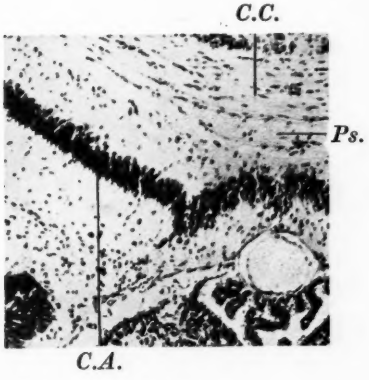

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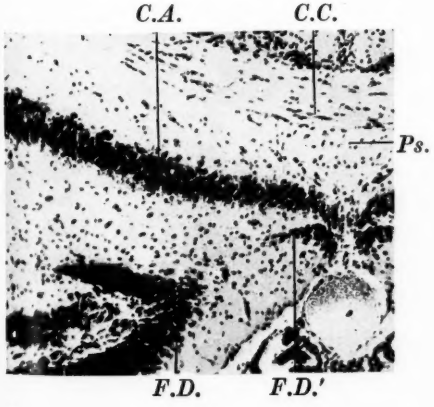

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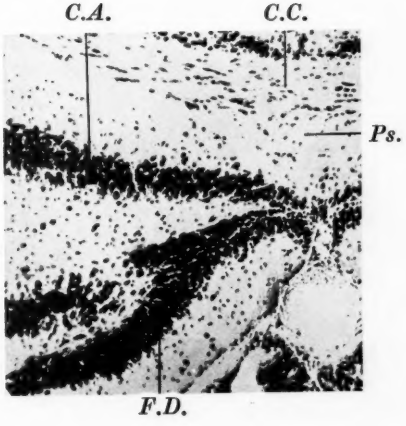

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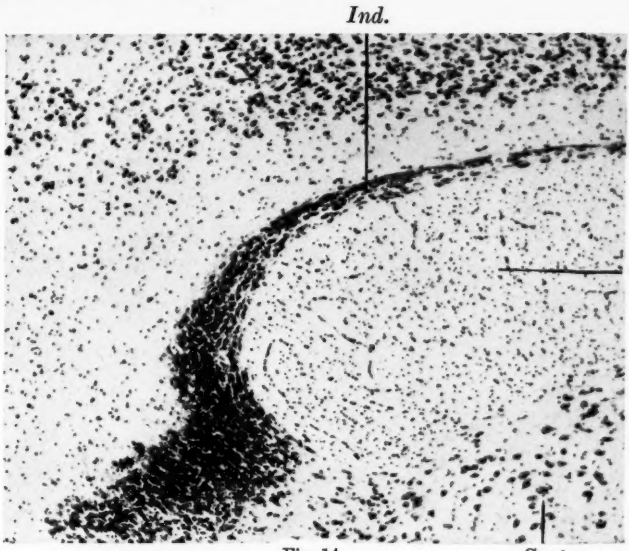

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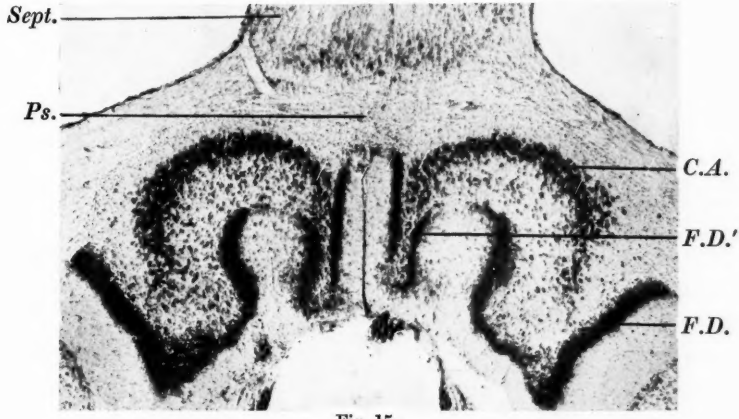

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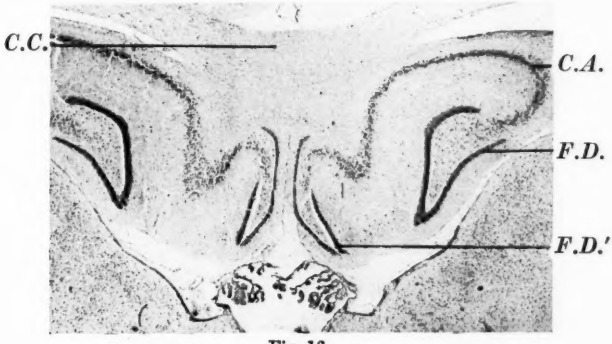

Fig. 16.

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CORTICOFUGAL DEGENERATION FOLLOWING THERMOCOAGULATION OF AREAS 4, 6 AND 4-s IN *MACACA MULATTA*[1]

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INTRODUCTION

Until recently only two precentral motor areas (areas 4 and 6 of Brodmann) have been recognized. Their functional and anatomical differences have been defined many times, and the course and destination of their projection fibres have already been described by various authors (Levin, 1936; Mettler, 1935; and Verhaart, 1938). From the physiological work of Hines (1936, 1937) and of Dusser de Barenne & McCulloch (1938) it is now apparent that between these two areas lies the so-called "strip area" of Hines—area 4-s of the latter authors —which has functions clearly distinguishable from those either of area 4 or 6 (Fig. 1).

In 1936 Hines observed that a narrow segment of precentral cortex about 3 mm. in width extending along the rostral border of area 4 (anatomically continuous with it) had specific physiological properties. Ablation of this area was followed by transient contralateral spasticity principally extensor in distribution; there was no forced grasping. An ablation restricted to area 6, in contrast, caused transient forced grasping (flexion), without extensor spasticity. Dusser de Barenne and McCulloch (1938) also differentiated this area (designating it "4-s", i.e. are 4-strip), from areas 4 and 6 by two procedures. (i) Local *strychninization* of 4-s suppressed the electrical activity of area 4, whereas strychninization of area 4 itself resulted in "firing" of this area, as evidenced by the appearance of "strychnine spikes" in its electrocorticogram (E.C.G.). Strychninization of area 6 caused spikes, not only in the E.C.G. of area 6, but in that of area 4 as well. (ii) *Electrical stimulation* of area 4-s temporarily suppressed the excitability of area 4, whereas stimulation

[1] This work was aided by a grant from the Knight Fund (M.A.K.), Yale University School of Medicine.

[2] Based on work carried out while the first author was a Rockefeller Foundation Fellow, 1938–9, in the Laboratories of Drs J. G. Dusser de Barenne and J. F. Fulton, Yale University School of Medicine.

of either area 4, or 6, had no such effect (Dusser de Barenne & McCulloch, 1939). Thus, it is possible by either of these two methods to define, at operation, the exact location of area 4-s. Dusser de Barenne & McCulloch have also shown that the suppression of electrical activity in area 4 by local strychninization of area 4-s is a functional disturbance operating over a "one-way" circuit—from area 4-s to the caudate nucleus, to the thalamus and thence to area 4. In contrast no functional pathway via the caudate has been discovered from area 6, or from area 4 itself.

On the basis of these physiological data it became desirable to investigate the problem of the anatomical connexions of these several regions by tracing the corticofugal degenerations following lesions so circumscribed as to lie entirely within each one. This is possible by the method of laminar thermocoagulation of Dusser de Barenne (1933), since this allows precise control of both the depth and the extent of each lesion.

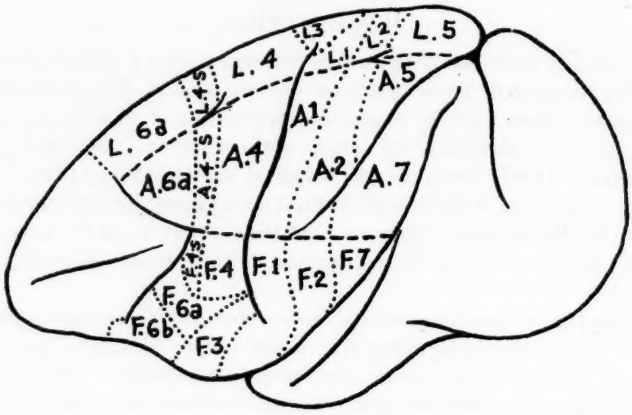

Fig. 1. Cytoarchitectural map of cortex of *Macaca mulatta* showing areas 6*a*, 4-s and 4 (after Dusser de Barenne & McCulloch).

I. METHODS

In each of eighteen macaque monkeys (*Macaca mulatta*) a small portion of one of the frontal areas was thermocoagulated through the entire thickness of the cortex. The arm region (area A. 4, two experiments; A. 6, six experiments; or A. 4-s, five experiments) was chosen in each instance, and, prior to coagulation, the exact location of A. 4-s was determined by the second physiological method of Dusser de Barenne & McCulloch (1938). Marchi degeneration was subsequently traced from each area. As controls lesions were made of areas 9–10–11–12 (two experiments) and of area 8 (three experiments) which are to be described elsewhere. Nine of the eighteen experiments are reported below in detail. In each experiment the cerebral cortex was exposed with sterile precautions, the region identified and the lesion made as just described. The animals were sacrificed between 14 and 18 days thereafter.

Physiological identification of area 4-s.[1] After exposure of the hemisphere two stigmatic electrodes were applied to the cortex, one for monopolar stimulation of a focus of area A. 4, the other, rostral to this for stimulation of a focus thought to be in area A. 4-s. Using a thyratron stimulator, repetitive stimuli were then applied for 4–5 sec. once every minute to the focus of area A. 4; stimuli were chosen of such a strength and character that uniform responses, usually extension of the wrist and fingers, were obtained over a period of 10 or 15 min. One stimulus was then applied to the presumed focus of A. 4-s. When this produced temporary suppression of the motor response from the periodic stimuli to area A. 4 it was certain that the second point was actually in area A. 4-s; this area was then thermocoagulated. Tracings of exposed cortex on cellophane with indication of the foci stimulated were made both before and after thermocoagulation.

Thermocoagulation. The thermocoagulation was carried out with a heated metal disk applied for a few seconds to destroy the cortex locally. A disk of 5×7 mm. was used for areas 4 and 6, one of 7×1 mm. for area 4-s (see Fig. 1).[2]

Preparation of Marchi sections. The animals were sacrificed under dial anaesthesia 14–18 days after operation by saline infusion through the left ventricle of the heart followed by 10% neutral formalin. The brain was immediately removed and fixed in formalin for 24 hr. After washing, it was placed in 3% potassium bichromate solution for a few days. It was then removed, and cut in frontal blocks which were fixed in bichromate for a week and subsequently impregnated with osmic acid after the method of Marchi. Two brains here described had received lesions of area A. 6 in the arm region only; four brains had similar lesions in area A. 4-s, and one had a lesion confined to A. 4. In addition, total ablation of area 6 by means of the usual gross surgical methods was made in two animals for the purpose of comparison of the fibre tract degeneration following this type lesion with those of the more discrete thermal destruction. The histological findings were as follows.

II. EXPERIMENTAL DEGENERATION

1. Lesions of area A. 4–s

Thermocoagulation of area A. 4-s, following physiological identification, gave the following results in the four monkeys studied:

Exp. 1. ***Thermocoagulation of area A. 4-s of right cerebral cortex; questionable degenerations to caudate and globus pallidus; clear degeneration in the thalamus, pontine nuclei and substantia nigra*** [T.C. No. 2].

Operation (18 *October* 1938). Under sterile precaution the right cerebral cortex of a young macaque (wt. 3·8 kg.) was exposed and two electrodes for monopolar stimulation placed, one on A. 4 and the other on A. 4-s, the indifferent electrode being in the rectum. By periodic stimulation of the A. 4 focus, 4 sec. once every minute, a constant minimal extension of the thumb and wrist was obtained. This response could be temporarily suppressed by applying a single 4–5 sec. stimulation to area A. 4-s. In this way A. 4-s was positively identified. Subsequently this area was thermocoagulated at 80° C. for 6 sec. (Fig. 2*B*).

[1] The physiological identification of area A. 4-s was made for us in each instance by Drs Dusser de Barenne and McCulloch, the procedure being carried out in their laboratory with the apparatus used in their investigations.

[2] Application of such a "thermode" at 80° C. for 5 or 6 sec. causes circumscribed destruction of the cortex over its entire thickness without damage to underlying structures (Dusser de Barenne, 1933).

***Autopsy* (31 *October*).** Prior to killing the animal, another similar stimulation experiment was carried out. It was found that stimulation of area A. 4-s inside the thermocoagulated region did not now result in suppression of the motor response obtained from the A. 4 focus. This was taken as evidence that this portion of area A. 4-s had been totally destroyed by the thermocoagulation on 18 October.

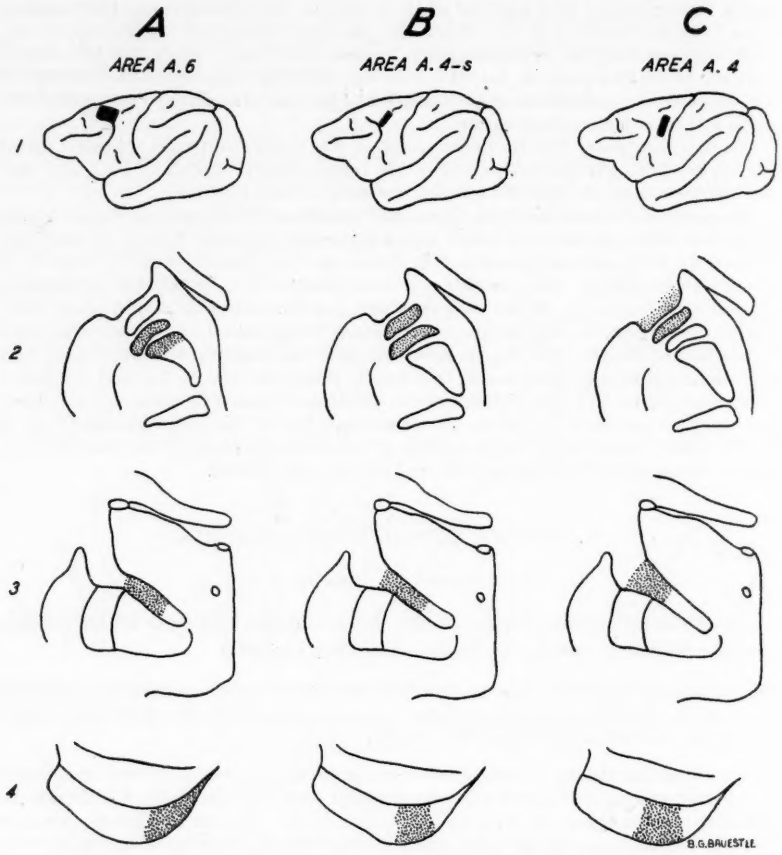

Fig. 2. Diagram of sites of lesions made by cortical thermocoagulation of areas A. 6*a* (1*A*); A. 4-s (1*B*) and A. 4 (1*C*) in *Macaca mulatta*; and of the location of degeneration from each of these lesions in the internal capsule and pyramid.

Marchi degeneration. Serial sections were made from the level of the lesion to the middle of the pons and sample sections from the medulla and spinal cord. Some blocks, however, were rather poorly impregnated so that the middle part of each could not be used. The outer parts showed a very distinct degeneration within the affected tract while pseudo-degeneration was almost totally absent. The *lesion* involved all the layers of the cortex, and underneath, in the white matter, a thin focus

of softening with a number of granular cells was present. From the lesion a distinctly impregnated group of degenerated fibres proceeded into the white matter of the convolution and could be traced to the corona radiata at a somewhat more occipital level, where the caput of the caudate nucleus was of about the same diameter as the putamen. The group of degenerated fibres reached the transition from the corona radiata to the capsula interna and separated in two bundles; one, the smaller, entering the corpus callosum, the other, about twice as large, entering the capsula interna. The latter group consisted of both thick and middle-sized fibres, which proceeded into the capsula and came near to the dorso-lateral margin of the caudate nucleus. The subcallosal area at this level contained a small number of very fine degenerated fibres, but none could be seen entering the caudate nucleus and the nucleus itself was clear.

At a more occipital level, where the diameter of the caudate nucleus was about half that of the putamen and where the frontal part of the globus pallidus appeared, the group of degenerated fibres occupied the dorso-lateral half of the internal capsule, but was mixed with a great number of undegenerated fibres, the osmic acid granulation of this region therefore being scattered. At the level of the anterior third of the thalamus and the tail of the caudate nucleus, the subcallosal layer still showed very fine degenerated fibres, but the caudate nucleus showed none. Some degenerated fibre bundles entered the inner part of the globus pallidus without spreading there. A small number of middle-sized degenerated fibres also spread and ran separately into the reticular nuclei of the thalamus. At the level of the anterior pole of Forel's field the bulk of the degenerated fibres within the capsula had shifted ventrally and medially; the bundle had diminished in size and the finer fibres, especially, were less numerous than in the more rostral slides. Everywhere in the thalamus, except in the medial nuclei, a diffuse degeneration of middle-sized fibres was found, not dense but rather distinct. In the zona incerta the same was the case, the fibres being somewhat thinner.

The cauda nuclei caudati and the subcallosal layer showed some indistinct degeneration. Within the inner part of the globus pallidus close to the capsula interna some degenerated fibre tracts were present. The region of the red nucleus and Forel's field was not well enough impregnated to make any conclusion possible. The corpus subthalamicus showed no degeneration.

Within the mesencephalon the degenerated fibre groups occupied the second fifth from the mid-line in the pes pedunculi, the rest being free from degeneration. There were quite a number of thick degenerated fibres and some middle-sized ones, but fine fibres could no longer be seen. Some of the fibres entered the stratum intermedium and probably the proximal part of the substantia nigra, but a greater number entered this substance at its caudal portion where they lay within it close to the degenerated region of the pes pedunculi and somewhat more dorso-laterally. At the boundary of the mid-brain and the pons the degeneration was found within the pedunculus lemnisci, both in the part close to the degenerated area of the pes pedunculi and in a somewhat more latero-dorsal part, but not in the medio-ventral portion. Fibres directly proceeding to the tegmentum mesencephali could not be found.

In the pons the fibres of the pedunculus lemnisci could not be found, the lemniscus medialis did not contain Marchi granulae, except some pseudo-degeneration on both sides. Within the longitudinal basal tracts of the right side there was a distinct degeneration, especially of thick fibres within the medio-dorsal bundles. In the caudal pontine level this degeneration had diminished considerably, but a severe degree of pseudo-degeneration made these slides less reliable.

In the medulla and the cervical segments of the cord no distinct degeneration in the pyramidal tracts could be found.

Exp. 2. *Thermocoagulation of left area A.* 4-*s; failure of A.* 4-*s to suppress A.* 4 14 *days later; uncertain striatal but clear-cut degenerations to thalamus, nigra and pons* [T.C. No. 3].

History. On 25 October 1939 area A. 4-s of the left cerebral hemisphere of a mature macaque (wt. 5·6 kg.) was identified and thermocoagulated as in Exp. 1. Slight right-sided motor paresis was evident for several days after operation, but no obvious spasticity. The experiment was terminated on 7 November, after absence of suppression of stimulation of the coagulated area was established.

Marchi degeneration. The sections were in part poorly impregnated; as in the previous experiment they showed the same distinct blackening of the degenerated fibres and almost total absence of pseudo-degeneration. The coagulated area was almost entirely limited to the cortex with a small granular-cell focus at the boundary of the white matter. The group of degenerated fibres taking their origin within this lesion proceeded to the white matter, the corona radiata and the capsula interna in the same way as in the first case. Part of the degeneration went to the corpus callosum, the subcallosal layer showing a very fine and rather indistinct granulation at the level of the caudal part of the caput nuclei caudati, but within the caudate nucleus no degenerated fibres could be distinguished with certainty. The group of degenerated fibres in the internal capsule occupied the same place as described previously; it shifted ventrally at the level of the frontal half of the thalamus, the fibres which occupied the most medial part of the capsule at more frontal levels having disappeared.

As in this series of slides the sections of the frontal part of the thalamus were better impregnated than in the preceding experiment the rearrangement of the degenerated fibres could be better appreciated. Just frontal to the frontal portion of the thalamus the degenerated fibres occupied the lateral half of the internal capsule, the medial half consisting of rather fine undegenerated fibres. At the frontal pole of the thalamus these fine fibres for the greater part shifted to the medial and somewhat to the dorsal side and seemed to form the greater part of the abundant quantity of myelinated fibres within the thalamus. At the same level the degenerated group shifted to a much more ventral part of the capsule, although they did not occupy its most ventro-medial portion. Many of the finer fibres among them also entered the thalamus, a fine granulation being found almost everywhere in the frontal parts of the thalamic nuclei. Some thicker and finer fibres, initially situated in a more dorso-lateral part of the capsule, shifted medially and somewhat dorsally, entering the thick fibre plexus close to the ventral border of the caudate nucleus, where the caput and the cauda meet. However, it was not possible to trace them in the caudate, although they came quite close to its boundary.

At a more caudal level, just frontal to Forel's field, there was another degeneration, found within the white matter of the convolutions, and the capsula externa and, at still more caudal levels, within the lateral part of the capsula interna, apparently not in connexion with the lesion of A. 4-s. It could be easily distinguished from this lesion because there was an undegenerated spot in the capsula interna separating the two. The degeneration from A. 4-s was found at a more occipital level in the second fourth of the capsula from the mid-line and within the mid-brain in the same portion of the pes pedunculi. Some of its fibres proceeded to the lateral and ventral parts of the thalamus at the level of Forel's field, no degeneration could be traced to Luys' subthalamic body or to the red nucleus. About the substantia nigra, the stratum intermedium, the pedunculus lemnisci and the longitudinal fibres of the basal pontine nuclei degeneration was found as in the first experiment. The medullary and spinal pyramids showed no distinct degeneration.

Exp. 3. *Thermocoagulation of area A.* 4-*s on left side; small accidental lesion adjacent. Degenerated fibres of passage in striated nuclei; clear degeneration to thalamus, nigra and pons* [T.C. No. 14].

History (23 *December* 1938). The left area A. 4-s of a mature female macaque, weighing 4·8 kg., was located by means of electrical stimulation as described in the previous experiments, thereafter a portion of A. 4-s, 7 × 1 mm. in extent, similar to that of the first two experiments was thermocoagulated at 80° C. for 6 sec. At the *autopsy*, 7 January 1939, there proved to be a coagulated spot of the size and shape seen in Fig. 2*B*. There was also another small damaged spot in the cortex just dorsal to the coagulation, close to the mid-line. Degeneration from the second accidental injury proved to be present throughout this series of slides, but was so distinctly separated from that from area A. 4-s that it could be ignored.

The group of degenerated fibres from the thermocoagulation could be followed through the white matter of the convolution to the corona radiata, where it divided in two, one proceeding to the corpus callosum, the other to the capsula interna. The degenerated fibres consisted largely of thick fibres while a relatively small number of middle-sized ones were also present. They reached the internal capsule at the level where the caudatum and the putamen are about the same size. At this level there was a very small number of thin granulae within the subcallosal layer. No degeneration could be traced into the caudate nucleus, although thick fibres passing through were found within the caudatum as well as putamen close to the junction of the capsula interna and the cerebral white matter. At the level of the frontal part of the thalamus the degenerated fibres were situated in the outer part of the pallidum within the capsula interna.

At the frontal level of H 2 and H the degenerated fibres began to shift to the more medial portion of the capsule, the finer fibres occupying the medial part in frontal levels and probably terminating there. At this level a small number of degenerated fibres enter the lateral and ventral thalamic nuclei as well as the zona incerta. There was some degeneration within H 2 and H, but no continuous degenerated fibres could be followed from the internal capsule to these systems. In more caudal levels the degeneration shifted to the second fifth from mid-line of the pes pedunculi, but the lateral boundary of the degeneration could not be determined because very scattered thick fibre degeneration occupied the whole lateral part except the bundle of Türck.

In the proximal level of the mid-brain some degenerated fibres entered the stratum intermedium and terminated in the substantia nigra; they could not be traced to the tegmentum mesencephali. Within the substantia nigra there was a large amount of deposit of reduced osmic acid outside the fibre tracts and the neurons; real fibre degeneration for this reason could not be studied within this nucleus.

Exp. 4. *Thermocoagulation of left area A.* 4-*s; degenerations similar to those of Exps.* 1–3 [T.C. No. 15].

History. The left area A. 4-s of a young macaque (wt. 3·2 kg.) was thermocoagulated after identification, as in the preceding experiment. The operation occurred on 13 January 1939 and the experiment was terminated 30 January 1939.

The Marchi slides showed a small but very distinct group of degenerated fibres emerging from the lesion and proceeding in the white matter of the gyrus. This passed caudally and medially, entering the white matter of the hemisphere and dividing in two groups. One group, chiefly consisting of middle-sized fibres that entered the corpus callosum and another, containing a greater percentage of thick fibres, were found. No degenerated fibres could be traced to the caudatum or the putamen, the subcallosal layer showed no distinct degeneration, but at the frontal level of the thalamus a number of fibres passed dorsally and medially and seemed to

enter the thalamus. At this level the group of degenerated fibres appeared opposite the lateral part of the pallidum.

At a more occipital level, just frontal to H 2, there was much pseudo-degeneration within the lateral part of the capsula interna, but the group of degenerated fibres could still be distinguished. A small number of them entered the reticular thalamic nuclei, at Forel's level, and the group shifted medially in the capsule at about this point. Within the pes pedunculi pseudo-degeneration made it impossible to determine the lateral border of the A. 4-s fibres, but it could be concluded that the most medial sixth of the pes was free from degeneration. A number of degenerated fibres entered the substantia nigra, especially in the caudal part, and a similar number entered the pedunculus lemnisci. Within the longitudinal fibres of the pes pontis a moderate scattered degeneration was found, but within the medullary and spinal pyramids no distinct degeneration was present.

2. Lesions of area A. 6

The lesions described in this section were placed in the upper part of area 6*a* (Vogt terminology), and being restricted to that portion of area 6 opposite to the motor arm area we refer to the region as area A. 6, i.e. arm 6, in conformity with the usage of Dusser de Barenne & McCulloch (1938).

(*a*) *Thermocoagulation*

In the first group of experiments, of which there are two, the lesions were made by the thermocoagulation technique.

Exp. 5. *Thermocoagulation of left area A.* 6. *No degeneration to caudate: heavy degeneration to thalamus, pons and especially to substantia nigra; no certain degeneration to cord* [T.C. No. 8].

History. An immature macaque (wt. 2·5 kg.) was operated upon on 4 November 1938. With the thermocoagulation apparatus at 80° C. and a thermode of 5 × 7 mm. coagulation of the anterior part of A. 6*a* of the left cerebral hemisphere was carried out lasting for 5·5 sec. Both at operation and after autopsy tracings were made of the coagulated spot (see Fig. 2*A*). As it was on the rostral part of area 6 there was no danger of damaging area 4-s which was not therefore delimited for fear of inducing stimulation degeneration. The experiment was terminated 21 November 1938, and Marchi slides were made.

Degenerations. A group of degenerated fibres, some thick and a greater number middle-sized, entered the capsula interna at the level at which the putamen is about half the size of the caudatum. At a more frontal level in which the putamen first appeared, the degenerated fibres lay within the bundles just dorsal to it. At the level where the putamen was about twice as large as the caudatum and where the pallidum showed its frontal tip, the centre of the degenerated group was opposite the medullary lamina between the putamen and the pallidum. The most medial part of the internal capsule, as well as the subcallosal layer, contained only a small number of very thin granules. No degenerated fibres to the caudatum could be found.

At the frontal part of the thalamus a small number of degenerated fibres proceeded medio-dorsally to the latero-dorsal part of the thalamus, but the greater proportion remained in the internal capsule and gradually shifted medially in it. The group of degenerated fibres occupied the region about the level of the internal medullary layer of the pallidum in a section somewhat frontal to the frontal end of H 2. At the level of this frontal end the fibres rather rapidly shifted medially and occupied the most medial third of the capsula interna at the level of the subthalamic nucleus, and the same part of the pes pedunculi within the mid-brain.

Within the pes pedunculi the thinner fibres were more numerous in the most medial part of the degenerated area, while the thicker ones were chiefly, but not entirely, found within its lateral part. Many fibres entered the substantia nigra in its medial third both at its proximal and its more caudal level. The medial third of the pedunculus lemnisci also contained a relatively large number of degenerated fibres, but at this level there was a considerable amount of pseudo-degeneration. In the medullary pyramid more granules were present on one side than the other, but a considerable pseudo-degeneration confused the picture. The cervical cord similarly showed more granules in its right lateral pyramid than on the left.

Exp. 6. *Small thermocoagulation lesion of posterior part of area A.* 6 (*left*). *No degeneration to caudate, questionable to putamen; all degenerations in capsule and peduncles more occipitally placed than in Exp.* 5 [T.C. No. 9].

History. The left cerebral hemisphere of an immature macaque (wt. 2·2 kg.) was exposed on 9 November 1938. In this experiment no electrical stimulation was performed, but a narrow vertically placed area, 1×7 mm., was thermocoagulated at 80° C. for 6 sec., at the boundary of areas A. 6 and L. 6. The animal was killed on 25 November 1938. Marchi slides were prepared in the usual way. At the autopsy a coagulated spot was found at the site traced during the operation, and there were also two minute lesions at the dorsal and ventral ends of the frontal part of area 4.

Degenerations. A small group of degenerated fibres entered the internal capsule at the level where the putamen is about twice the size of the caudatum. No degenerated fibres could be traced to the caudatum, but the degenerated group was chiefly situated at the putaminal side of the capsule, and some degenerated transition fibres were found within this nucleus close to the capsule. In more occipital levels nothing distinct could be seen. Very few fibres could be traced within the reticular area of the thalamus and none to the red nucleus. Some went to the zona incerta and a few to the substantia nigra and the pedunculus lemnisci. The pes pedunculi contained degenerated fibres throughout, but *fine* fibres were present in the most medial sixth only. This case showed thus that the fibres from the more occipital part of A. 6, as compared with those of the previous experiment, reach the internal capsule at a somewhat more occipital level and within the pes pedunculi occupy its most medial sixth.

(*b*) *Ablation of area* 6

In connexion with the foregoing experiments area A. 6 was actually ablated in two instances by blunt dissection, instead of being thermocoagulated. By this means much larger tracts of degeneration were obtained. The following are the protocols of these two experiments.

Exp. 7. *Ablation of left area A.* 6. *No degeneration to caudate nucleus, fibres of passage in putamen; heavy degeneration to thalamus, reticular formation substantia nigra and pons; no definite fibres in pyramids, or spinal cord* [T.P. No. 4].

History. In an immature macaque (wt. 2·2 kg.) the left hemisphere was exposed on 31 October 1938, and part of area A. 6 situated medial to the arcuate sulcus was extirpated in one block and the animal killed for histological examination on 16 November 1938.

Degeneration. At the site of the ablation there was a deep softening of the white matter of the gyrus, and from this lesion a rather large group of degenerated fibres proceeded to the corona radiata, the capsula interna and the corpus callosum in almost exactly the same way as in Exp. 5, in which a similar lesion was made by thermocoagulation. But in the present case the group of degenerated fibres was somewhat larger. No fibres could be traced to the caudatum, but there were some degenerated fibres passing through the putamen. No fibres proceeded to the red

nucleus, but some went to the frontal thalamic nuclei and some to the reticular and lateral ones at a level somewhat frontal to Forel's systems. Within the pes pedunculi the degenerated fibres entered the substantia nigra and the pedunculus lemnisci in the usual way. The medullary pyramid showed only a small number of granules.

Exp. 8. *Ablation of area A.* 6*; heavy degenerations as in Exp.* 7 [T.C. No. 7].

History. From a macaque of size (wt. 2·6 kg.) similar to that of Exp. 7 the left cortex was exposed on 2 November 1938 and a larger portion of area A. 6 was removed; the lesion extending somewhat farther occipitally than in the preceding experiment, but it was also confined to the part of area A. 6 situated medially to the arcuate sulcus. The animal was killed 18 November 1938.

Degenerations. At the site of the lesion the white matter of the gyrus was softened to about 5 mm. dorsal to the caudatum. There was a rather large group of degenerated fibres, proceeding in the usual way to the capsula interna and the corpus callosum. The subcallosal layer showed a number of fine granules at the level of the lesion and farther occipital to the level where the most frontal part of the thalamus appeared. In the frontal part of H 2 and the ansa lenticularis a number of fine fibres were found, which passed to the zona incerta and Forel's field H, but in the more occipital parts of this field they were no longer present. The frontal capsule of the red nucleus did not show any distinct degeneration, and at this level the degenerated group within the capsule had reached its most medial part. Within the pes pedunculi this group occupied the medial third, the finer fibres chiefly being found toward the mid-line in this third, but even the big granulae were not absent here. The degenerated fibres entered the reticular nuclei of the thalamus, the stratum intermedium of the substantia nigra and the medial half of the pedunculus lemnisci. They were found chiefly in the distal half of the substantia nigra. No degeneration was seen within the mesencephalon and the pons, dorsal to the mesial fillet.

Within the pes pontis degeneration was found chiefly in the medio-dorsal half of the longitudinal fibres, but in the medulla only a few degenerated fibres were present in the pyramid, most of the basal pontine fibres therefore ending at the pontine level.

One tract in Exp. 8 must be mentioned, although it does not show any degeneration. It consists of two small bundles leaving the capsula or some other fibre group at the frontal level of the subthalamic nucleus. Then they are situated at the medio-dorsal border of the subthalamic nucleus, proceeding caudally along the dorsal border of the substantia nigra, where they gradually seem to lose their fibres and dissolve in this substance, as they are not found at a more caudal level. These may be the fibres mentioned in Levin's paper as belonging to the cortico-mesencephalic tegmental tract which he found degenerated when the prefrontal region was ablated (Levin, 1936, Exp. 11). In his case it was larger and could be followed into the mesencephalic tegmentum. Most of the fibres from both tracts were lost at the dorsal border of the substantia nigra, and it is possible that Winkler is right when he attributes to this bundle, which he described in humans as bundle X, a chiefly striato-fugal character. It is definitely an inconstant bundle and belongs to the so-called aberrant fibres or bundles that occur especially in the systems of the pes pedunculi. This could be thought of as an aberrant strio- and pallido-nigral system that occasionally can contain fibres from the frontal lobe, but as fibres of aberrant bundles frequently return to their usual area and cannot

then be followed, it is not possible to conclude that such tracts end at the place where they can no more be seen.

Compared with the results after thermocoagulation of A. 4-s, the degeneration following the lesions of area A. 6 was situated more frontally in the white matter of the hemisphere and more medio-ventrally within the internal capsule frontal to the level of Forel's systems, while in more caudal levels and in the pes pedunculi it occupied the most medial third of capsula and pes. As in the previous cases the degeneration occupied the second fifth or fourth from midline of the pes pedunculi; the fibres from A. 6*a* were situated more medially, but there was a rather extensive overlapping, as even in the cases with only partial coagulation of the different areas an overlapping of the medial third and the second medial fifth or fourth took place. The same was the case within the internal capsule, but there no such exact measures could be given, as the lateral boundary of the capsule was vague and depended greatly on the direction of the cutting of the section.

The last experiment in particular gives evidence that the most medial fibres of the frontal part of the internal capsule reach neither the occipital part of the capsule nor the pes pedunculi, since frontal to the level of Forel's tract a great many fibres were present medial to the degenerated group and, caudal to that level, the degenerated group occupied the most medial part.

3. Lesions of area A. 4

Lesions of area 4 (arm region) by the thermocoagulation method were made in two experiments of which one is described. Since the results of ablation have been fully described by other authors (Levin, 1936; Fulton & Sheehan, 1935, etc.), no ablation experiments will be reported here.

Exp. 9. *Local thermocoagulation of area A.* 4. *No degeneration to striatum or red nucleus; heavy tracts to thalamus, substantia nigra and spinal cord, etc.* [T.C. No. 10].

History. The left hemisphere of an immature male macaque (wt. 2·2 kg.) was explored on 11 November 1938. The arm region of area 4 was coagulated at 80° C. for 6 sec. After the coagulation an area 5 mm. wide and 7 mm. long appeared markedly blanched with sharp boundaries in the middle of the precentral gyrus (Fig. 2*C*). The animal was killed on 28 November 1938.

Degenerations. Marchi degeneration found within the grey matter of the hemisphere closely caudal to the lesion was divided in two parts; one group of fibres entered the corpus callosum near the subcallosal layer and some fibres reached its border, but no degeneration was found anywhere within this layer. The other group entered the capsula interna which was filled with degenerated fibres at the most frontal level of the globus pallidus; the degenerated tract consisted chiefly of thick fibres, which were more numerous than in the previous cases of lesions confined to area 6 or area A. 4-s. Everywhere within the capsule the degenerated group occupied a somewhat more lateral place than in the previous experiments. At the level of the frontal part of the thalamus fibres were found chiefly between the putamen and the caudate, while following coagulation of area A. 4-s the degeneration was situated between the outer part of the pallidum and the caudatum and, in the area 6 experiments, between the internal medullary lamina and the thalamus. No degenerated fibres could be traced into the caudate nucleus or the putamen. Some entered the

reticular nuclei of the thalamus especially just frontal and dorsal to the frontal part of H 2. At the level of H 2 and the frontal portion of the red nucleus, the degeneration was distinct and hardly any pseudo-degeneration was present; it could, therefore, be said definitely that no degenerated fibres entered H 2 or passed to the red nucleus. Within the occipital levels of the capsula interna the group of degenerated fibres shifted to ventro-medial, but did not reach the medial part; in the pes pedunculi it occupied the second and third sixth from the mid-line. Tracts of area 4 therefore reached less far medially and farther laterally than did those from either of the two other regions, but all overlapped to a greater or lesser extent.

Many thick degenerated fibres were seen entering the substantia nigra at all its levels, some entered the pedunculus lemnisci but could not be traced farther dorsal than the mesial lemniscus. Within the pontine and the medullary pyramid a number of thick degenerated fibres were present, the greater number of which crossed in the pyramidal decussation to the lateral tract while a much smaller number proceeded to the ipsilateral lateral column.

III. DISCUSSION

Within the limitation of the Marchi method it has been possible to identify various degenerated fibre tracts from three physiologically established areas of the precentral cortex (areas 6, 4-s, and 4). As was expected, no unknown cortico-fugal projections have been discovered, but the more generally accepted fronto-subcortical connexions have been reaffirmed. To the above information have been added three findings: (i) that fibres pass from *each* of these three areas to *all* the regions cited below, i.e. thalamus, substantia nigra, pons, etc.; (ii) that no fibres were found passing from one cortical area to any subcortical region to which fibres from the other two cortical areas did not also pass; (iii) that in the subcortical paths and nuclei into which these tracts enter there is a precise topical arrangement of the fibres from each area.

Cortico-pontine fibres. The greatest bulk of fibres passing from area 6 and area 4-s, and also many fibres from area 4, pass to the pons and end in its basal nuclei. When area 6 was damaged, as in Exps. 5–8, the most medial part of the pes pedunculi (area of Arnold's bundle) showed considerable degeneration, although in none of the cases was the degeneration confined to that area. The pedunculus lemnisci emerged from almost the whole extent of the pes pedunculi at the transition of the mid-brain and the pons, and indicated the border between the substantia nigra and the basal pontine nuclei. Its fibres probably end largely within those pontine nuclei situated around, and within, the medial part of the mesial fillet. Some fibres can be seen proceeding through the mesial fillet to the tegmentum pontis, but a thick tract proceeding in this way is rarely seen. Since in these cases the greater part of such tracts in lower pontine levels reverses and joins the pyramid in the pes pontis, it is possible that the functional significance of this pedunculus lemnisci has been overestimated, and that most of its fibres may be looked upon as ordinary fibres of the pes pedunculi.

Distinct cortico-tegmental and bulbar fibres, mentioned by Levin, have not been found consistently in our series; they are rare and are seen to occur only in association with massive degeneration of the pyramidal tract.

Cortico-nigral fibres. In all cases a number of fibres terminated within the substantia nigra. These emerged from the pes pedunculi at almost all levels of the mid-brain, but frequently the caudal part seemed to receive them most conspicuously.

Cortico-thalamic system. Especially in the frontal part and at the level somewhat frontal to Forel's systems, fibres were seen emerging from the capsule to the reticular and lateral thalamic nuclei. The rapid disappearance of the finer fibres within the medial part of the capsule at the frontal part of the thalamus indicates, as pointed out by Levin (1936), that the frontal areas send still other fibres to the thalamus.

Cortico-rubral fibres. Our findings agree entirely with the painstaking description of Levin (1936), i.e. no evidence of a distinct cortico-rubral system was found in our sections, and it is certain that no fibres from the frontal lobe extend to the magno-cellular part of the red nucleus.

Cortico-mesencephalic-tegmental tract. As noted above, the existence of this tract is not established. The bundle pointed out by Levin may be an aberrant tract only traceable along the dorsal border of the substantia nigra, which occasionally carries a number of lenticulofugal fibres (Winkler, 1929). Or the degenerated fibres seen by Levin may have derived from a prefrontal area, which was not damaged in any instance in this series.

Cortico-subthalamic fibres could be seen only rarely emerging from the capsule at the level of H2 and reaching as far as the zona incerta. No degeneration to the subthalamic body was traced.

Cortico-striate system. As Levin (1936) points out, the opinion of various authorities differs widely with regard to this system. Following extensive lesions in the prefrontal region, he observed degeneration within the subcallosal layer only. "No degeneration of fibres could be seen in the substance of the caudatum itself", he writes, "but of course this nucleus contains few and very thin myelinated fibres." We can agree with him, for no degeneration could be seen in any of our cases although the caudatum is known to contain many very thin myelinated fibres. Lesions in the caudate nucleus and in the putamen, made by one of us (W.J.C.V.) for other purposes, showed a distinct degeneration within these thin fibres when impregnated with osmic acid, and therefore it seems obvious that they *originate* in the caudate nucleus and that no degeneration can be caused in them by lesions in the cortex. Degenerated fibres splitting off from the capsula interna and passing through the striate nuclei were, however, found in many of our cases, within both the caudatum and putamen.

Our slides similarly do not permit any conclusion about cortico-pallidal connexions. These connexions are generally admitted, but certainly a part of the degenerated fibres seen within the pallidum in such cases are "fibres of passage". In lower mammals, even in carnivores, the innermost part of the globus pallidus is situated partly within the capsula interna. In higher primates and man it is more isolated, but still a part of the capsule takes its course through the pallidum—also fibres of passage.

Connexions between area 4-s and the caudate nucleus have been so definitely established by the physiological experiments of Dusser de Barenne & McCulloch (1938) that it is disappointing not to have found degenerating fibres from this region entering into and ending in the caudate nucleus. Collaterals of cortico-spinal fibres entering the caudate nucleus have been described by Ramón y Cajal (1909), but it is not known in which area of the cortex they originate. These fibres are very fine, and have not as yet been identified by the Marchi method even by other investigators using large cortical lesions. Our method, in which very small lesions are made, is not adapted to a search for such tracts consisting of few and thinly myelinated fibres. It should be pointed out that the apparent discrepancy between the findings in the degeneration experiments and those of Dusser de Barenne & McCulloch may readily be explained by the fact that the Marchi degeneration does not show the actual ends of the degenerated axons, but stops where the myelin sheath ends, often several millimetres before the actual axonal termination.

Cortico-spinal tracts. Few degenerated fibres were found in the spinal pyramidal tracts following lesions of either areas 4-s or 6, whether the lesions were made by thermocoagulation or by ablation. This is contrary to previous findings of one of us, of distinct, though slight, degeneration in these tracts following ablation of area 6 (Kennard, 1935). In the present series all the lesions, even those made by ablation, were smaller than those made in the earlier experiments just mentioned. The discrepancy may then be accounted for in one of two ways: (i) either the present lesions were too small to cause damage to a sufficient number of cortico-spinal fibres to be recognized as degenerated in the tracts, or (ii) the lesions made in the first series went deep enough to injure tracts from areas other than area 6. Further investigation of this matter is in progress. The failure to find cortico-spinal degeneration from the strip lesions need not, therefore, preclude the existence of a small cortico-spinal projection from this region.

Arrangement of fibres. The special character of our experiments justifies a further statement concerning the fronto-subcortical connexions. In the first place, no connexions taking their origin from area A. 4-s differed in character from those arising from areas 4 or 6, i.e. all these tracts passed to the same nuclei, *but not to exactly the same part of those nuclei* (Fig. 1). Most frontally lie the fibres originating from area 6 (Exps. 5–8) which enter the capsula interna at the level where the most frontal part of the putamen appears. At the most frontal level of the thalamus they are found within that part of the capsule situated between the inner part of the pallidum and the thalamus. They shift rapidly medially within the capsule somewhat frontal to the level of Forel's systems and reach its *most medial* part at the level of H2. Within the pes pedunculi they occupy the *most medial third* approximately, and even rather thick degenerated fibres are found there.

The degenerated fibres originating in area A. 4-s (Exps. 1–4) reach the capsula interna, somewhat more occipitally, at the level where the putamen

is almost of the same size as the caudatum. At the most frontal levels of the thalamus the degeneration is found near the part of the capsule where the inner medullary lamina of the pallidum appears. It shifts rapidly medially at the same level as the others, but does not reach the innermost part of the capsula interna or of the *pes pedunculi*. *Within the pes pedunculi* fibres are found in its second fifth or fourth part from the mid-line, the most medial part showing no distinct degeneration.

The fibres from area A. 4 (Exp. 9) reach the capsule at the level where the pallidum shows its most frontal part. At the level where the thalamus appears they are found opposite to the outer part of the pallidum and shift medially at a more caudal level, *but do not reach the innermost* part of the capsule. Within the pes pedunculi they are situated in the lateral two-thirds of the medial half, or the medial half of the pyramidal area. Their topographical place within the pes pedunculi therefore indicates the interrelation in the capsula interna and also the interrelation with respect to their ending in the different subcortical nuclei, as was pointed out for the thalamus by Droogleever-Fortuyn (1938) for the substantia nigra and the basal pontine nuclei by Winkler (1929). It must therefore be concluded that the different fibres, although entering in the same subcortical nuclei, send their endings to different parts of those nuclei, the fibres originating in a more frontal level finding their place in a more medio-ventral part of the capsula interna and a more medial part of the pes pedunculi, and ending in corresponding parts of the already mentioned subcortical centre.

Within the capsula interna and the pes pedunculi there is an important overlapping: the fibres from area A. 6 occupy the medial third, those from A. 4-s the second medial fifth or fourth, and those from A. 4 the second and third medial sixths in the pes pedunculus. The real overlapping, however, is much greater, as in our cases only the arm portion of each of the three areas was injured and the fibres from the leg part were not degenerated; these naturally find their place between the others.

CONCLUSIONS

1. Following identification of areas A.6, A.4-s, and A.4 (A. = arm region), laminar thermocoagulation within each region will produce Marchi degeneration localized to fibre tracts from each region; these have been traced to their subcortical destinations.

2. All tracts previously well established by other authors for the precentral gyrus as a whole were found in the present investigation for the individual areas and no new connexions were established.

3. Fibres from areas A.6, A.4-s and A.4 were found ending chiefly in basal pontine nuclei, substantia nigra and thalamus; no fibres could be traced to the red nucleus.

4. The existence of cortico-caudate fibres was not definitely established.

This statement does not invalidate the findings of the physiological experiments of Dusser de Barenne & McCulloch, since the Marchi method does not reveal the actual axonal endings of the degenerated neurons.

5. With the exception of the cortico-spinal tract from A.4 no tract was found in any one cortical area which did not appear in the other two (6 and 4-s) as well.

6. The fibres from each area are arranged in a definite pattern throughout their course in internal capsule; and they have a similar topical organization in the pes pedunculi, and as they project upon the various subcortical nuclei.

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THE INNERVATION OF THE MUSCULATURE OF THE TONGUE

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THE innervation of the musculature of the tongue has been the subject of much investigation from the histological and physiological points of view. Controversy has ranged around the questions of proprioception and the autonomic innervation of the musculature in this organ. These problems have been reinvestigated by the application of a modified intravital staining method. The results of histological observations made on normal and operated animals have been correlated with clinical findings in man. Nerve stimulation experiments have also been carried out in order to determine the presence or absence of afferent fibres in the hypoglossal nerve.

MATERIAL AND METHODS

The material and experimental procedures which were employed are summarized in Tables I and II.

Table I. *Animal material*

Animals and no. of cases	Muscles studied	Operation	Days allowed for degeneration
34 rats	Intrinsic musculature of tongue	Nil	—
3 rats	Genioglossus and hyoglossus muscles	Nil	—
2 rats	Intrinsic musculature of tongue	Right hypoglossal nerve cut at base of skull proximal to its junction with branches from superior cervical ganglion	5
3 rats	Do.	Right hypoglossal nerve cut close to its point of entry into the tongue	12
2 rats	Do.	Right cervical sympathetic chain resected	12 and 13
1 rabbit	Do.	Do.	74
1 rat	Do.	Right hypoglossal nerve cut close to the tongue and right cervical sympathetic chain resected	12
1 rat	Do.	Right hypoglossal and lingual nerves cut on the hyoglossus muscle and right cervical sympathetic chain resected	12
1 rat	Do.	Right lingual nerve sectioned	12

Table I (*continued*)

Animals and no. of cases	Muscles studied	Operation	Days allowed for degeneration
1 rat	Intrinsic musculature of tongue	Right lingual nerve sectioned and right cervical sympathetic chain resected	12
1 rat	—	Stimulation of the right hypoglossal nerve before and after removal of the right superior cervical ganglion	—
1 rat	—	Stimulation of the right hypoglossal nerve. Stimulation of the left hypoglossal nerve after complete isolation from surrounding tissues	—

Table II. *Clinical material*

		Tests performed		
No. of cases	Nature of experiment	Taste on the anterior two-thirds of the tongue by application of cane sugar, common salt and quinine sulphate	Proprioception in the tongue by deformation and stretch	Pain and touch in the anterior two-thirds of the tongue, lower gum and lip, by pin-prick and v. Frey hairs
15	Unilateral and bilateral infiltration of lingual and inferior alveolar nerves with 2% novocain and 0·01% adrenalin	+	+	+
3	Painting surface of tongue with 2% "decicain"	–	+	–
1	Spraying buccal cavity with 5% cocaine	–	+	–
1	Central root section of right trigeminal nerve	–	–	+ including upper lip and gum

STAINING TECHNIQUE

In the course of studies on the innervation of the skin of the ear of the rabbit (Woollard, *et al.* 1939), it was found that nerves could be distinguished *in vivo* after subcutaneous injection with solutions of methylene blue. The staining process is influenced by a variety of factors, chief among which are the concentration and quantity of the dye solution injected and the period elapsing between introduction of the dye and observation of the tissue. Relatively small deviations from the conditions for optimal staining frequently lead to poor results. In the case of the tongue, direct observation *in vivo* cannot be employed, and it becomes necessary to remove the tissue at a suitable time after intramuscular injection of the dye to prepare it for histological examination. The time factor thus assumes greater importance than

in the case of the ear, in which the point of optimal staining can be determined by direct observation.

Experiments. Adult rats under ether anaesthesia were used. A measured quantity of methylene blue solution was injected into the tongue, and after a known period of time the animal was killed by opening the thorax and the tongue excised. The epithelium was removed and thin slices of muscle were transferred to 8 % aqueous ammonium molybdate. The specimens, after clearing, were mounted as whole preparations.

The methylene blue solutions were prepared by dissolving the dye in 0·9 % sodium chloride solution. On account of the relatively low aqueous solubility of methylene blue at room temperature and the difficulty in detecting undissolved material, care is necessary in preparing solutions of definite strength. This difficulty was overcome by gently warming the dye and saline solution on a water bath for a few minutes and subsequently checking the strength of the solution colorimetrically against a standard solution of the dye. Experiments in which dye solutions prepared at room temperature were compared with solutions which had been heated in the above manner showed that the heating process had no detectable effect on the staining qualities. Although in most experiments B.D.H. methylene blue (Standard Stain) was used, similar results were obtained using "Methylenblau med. pur." of Dr G. Gruebler and Co., Leipzig.

The first three series of experiments were designed to determine the influence on the staining process of the concentration of the dye and the period elapsing between introduction of the stain and removal of the tongue (survival period). The volume injected was 0·65 ml. In the first series of experiments the animals were allowed to survive for 15 min.; in the second series the survival period was 30 min. The concentrations of dye tested were 0·005, 0·01, 0·02 and 0·03 %. In all these experiments the staining of nerve was satisfactory, but in certain other respects the results differed considerably. Staining of neurolemma sheath cells, muscle fibres, precipitation and the ingestion of dye particles by macrophages became more marked with increasing concentration in both series. Reticular connective tissue was sometimes stained more markedly when higher concentrations were used. Motor end-plates and fine fibres to blood vessels were well stained by 0·01 and 0·02 % solutions of the dye in both series. The best results were obtained using a 0·01 % solution of methylene blue with a survival period of 15 min., under which conditions the muscle fibres were only slightly stained, nerves were well stained, motor end-plates showed clearly, and fine fibres to blood vessels were seen in abundance.

The third series of experiments was performed in order to determine whether better results could be obtained by using 0·65 ml. of 0·01 % methylene blue solution for a survival period slightly longer or shorter than 15 min. This was not found to be the case.

In a fourth series of experiments smaller volumes were used with a survival

period of 15 min. Concentrations of methylene blue of 0·005, 0·01, 0·02 and 0·03 % in quantities of 0·15 ml. and 0·01 % in quantities of 0·4 ml. were tested. The best results were again obtained with 0·01 % solution.

The results obtained with smaller quantities were, however, inferior to those in which 0·65 ml. were used. In experiments using small volumes it was difficult to observe fine fibres, and it would appear that in order to stain these fibres consistently it is essential to use the larger dose. It was therefore decided in the present investigations to employ 0·01 % solutions of methylene blue in 0·9 % sodium chloride with a survival period of 15 min. Single doses of 0·65 ml. were used. Since only relatively small areas are stained by this amount, 0·65 ml. were introduced into two or more sites when it was desired to study the whole of the musculature of the tongue.

Whilst this technique gives consistently good results for the tongue, suitable modifications must be made when staining nerves in other organs and tissues.

OBSERVATIONS

The innervation of the intrinsic musculature

The intrinsic musculature of the normal tongue is profusely innervated (Pl. I, fig. 1). The bundles of the hypoglossal nerve consist of many thick and a smaller number of thin fibres. Most of the thick fibres on leaving the bundles can be traced to motor end-plates. Others (Pl. I, fig. 2) become gradually thinner, often assume a varicose appearance and finally end on blood vessels (Pl. II, fig. 3). The thin fibres, which are frequently varicose, also end on blood vessels. The motor end-plates never receive more than one nerve fibre (Pl. II, fig. 4), and there are no accessory motor nerve endings. No sensory endings of any kind were seen, nor were any nerve cells observed along the course of the nerve bundles in the tongue.

Circular muscle fibres on arterioles sometimes stain deeply for short distances. The histological picture so produced (Pl. IV, fig. 10) may bear a spurious resemblance to a neuro-muscular spindle (Pl. III, fig. 9; Pl. IV, figs. 11, 12). The true nature of these structures is revealed by the details of their innervation.

Section of the hypoglossal nerve close to the tongue leads to degeneration of all the nerve fibres ending in the muscles and on blood vessels. Section of the hypoglossal nerve at the base of the skull proximal to its junction with branches from the superior cervical ganglion leads to degeneration of the motor fibres but does not affect the nerves supplying the blood vessels. In such a case, the undegenerated sympathetic fibres can be seen coursing among the degenerated fibres of hypoglossal origin (Pl. II, fig. 5). The sympathetic fibres frequently intertwine with one another (Pl. II, fig. 6) whilst on their way to blood vessels. There are no nerve nets. The larger sympathetic fibres have thin myelin sheaths which are lost as the fibres become finer in calibre. Unilateral

removal of the cervical sympathetic chain leads to degeneration of the fibres supplying the blood vessels on the operated side.

Meshworks of fine fibres (Pl. III, fig. 7) are sometimes observed in relation to all the cellular structures present; their configuration suggests a nervous origin. They are, however, devoid of sheaths and cannot be traced into continuity with nerve trunks; moreover, they persist on complete denervation. They are therefore considered to be reticular connective tissue. The arrangement of these fibres bears considerable resemblance to the sympathetic ground plexus, periterminal network and terminal reticulum of some continental authors.

Nerve stimulation experiments

The right hypoglossal nerve was exposed in a rat under light ether anaesthesia. The nerve was stimulated by pinching and by condenser discharges. Stimulation which produced tetanic contraction of the intrinsic and extrinsic musculature of the tongue led to slight dilatation of the right pupil, which rapidly returned to its former size on interruption of the stimulus. The left hypoglossal nerve was then exposed and carefully freed from all surrounding tissues as far as its exit from the base of the skull. During the course of the dissection fine nerve filaments from the region of the superior cervical ganglion to the hypoglossal nerve were severed. Stimulation of the nerve then gave rise to no dilatation of the pupil.

In another rat stimulation of the right hypoglossal nerve gave rise to dilatation of the pupil, but this reaction was not obtained after removal of the right superior cervical ganglion.

Clinical findings

In cases in which the lingual and inferior alveolar nerves were anaesthetized, the area of ageusia was limited to the anterior two-thirds of the tongue and bounded by the midline. The area of anaesthesia to pain and touch was limited by the midline on the tongue and lower lip, but extended for a variable distance as far as the lateral incisor tooth on the lower gum. These findings were confirmed in a case of unilateral central root section of the trigeminal nerve. In this case it was also established that anaesthesia was limited by the midline on the upper gum and lip.

Bilateral anaesthetization of the lingual and inferior alveolar nerves led to the abolition of all forms of sensation tested in the anterior two-thirds of the tongue. The tongue was not, however, ataxic, though articulation was difficult. There was no tendency to bite the tongue. These results were confirmed in the cases in which "decicain" and cocaine were applied to the mucosa.

The clinical results suggested that a certain degree of proprioception might be mediated by sensory endings at the base of the tongue.

The sensory innervation of the extrinsic musculature

The hyoglossus and genioglossus muscles of the rat were examined for sensory nerve endings. These were not found in the fleshy parts of the muscles, but, at the attachment of the genioglossus muscle to the mandible, endings (Pl. III, fig. 8) were found which consist of a thick coil of axis cylinder closely surrounded by enlarged neurolemmal cells. The fibre eventually leaves the coil and, after pursuing a linear course for a short distance, terminates in a small swelling. The configuration of these endings suggest that they respond to changes in tension.

DISCUSSION

Whilst many authors claim to have demonstrated neuro-muscular spindles in the intrinsic and extrinsic muscles of the tongue in animals, in no case is the evidence convincing. The possibility of their existence in man, however, cannot be excluded.

Pilliet (1890), working on a large number of unspecified mammals, found neuro-muscular structures very rarely in sections of the tongue. The author, however, did not differentiate between Pacinian corpuscles and neuro-muscular spindles, and the method of staining is not indicated.

Franqué (1890) records the finding of a muscle spindle with seven muscle fibres in a section of the human tongue. Forster (1894) studied sections of human hyoglossus, genioglossus and omohyoid muscles stained in alum carmine and eosin, and many muscle spindles were stated to occur in these muscles. Schaffer (1922) in his text-book illustrates a transverse section through a muscle spindle in the human tongue.

Langworthy (1924*a*, 1924*b*), using methylene blue, found in the cat, pig, dog, opossum and rat, neuro-muscular spindles in the intrinsic and extrinsic muscles of the tongue. No illustrations of neuro-muscular spindles are given and the criteria for their identification are based on the descriptions given by Sutton (1915) in a study entitled "On the development of the neuro-muscular spindle in the extrinsic eye muscles of the pig." Sutton's figures of methylene blue preparations depict structures similar to stained circular muscle fibres around arterioles. It is now generally accepted that there are no neuro-muscular spindles in the extrinsic ocular muscles.

Tarkhan (1936*a*), examining tongues of rabbits prepared by the method of Bielschowsky, found muscle spindles in the intrinsic musculature of the tongue. Consideration of both the author's photomicrographs and text shows that the preparations did not allow of the identification of the innervation of the structures believed to represent neuro-muscular spindles. The only grounds apparent for the statement that the muscle fibres depicted belong to the class of neuro-muscular spindles is their somewhat fusiform shape and the small diameter of the component muscle fibres. In the absence of the demonstration of the nerve endings the interpretation of fusiform

groups of muscle fibres as neuro-muscular spindles must be accepted with caution.

In contradistinction to the above-mentioned authors, the following workers have recorded the absence of neuro-muscular spindles in the intrinsic muscles of the tongue: Sherrington (1894; cat and monkey; gold chloride and non-specific stains on sections), Batten (1897; man; non-specific stains on sections and Sihler's method on teased preparations), Cipollone (1897; man), Baum (1900; man, numerous mammals, amphibia and fishes; gold chloride and non-specific stains on sections), Boeke (1927; mouse, rat and hedgehog; Bielschowsky's silver impregnation), Hewer (1935; human foetuses; silver pyridine), Boyd (1937; adult and foetal rabbits; various silver methods and methylene blue), Carleton (1937; rabbit, chameleon and ant-eater; pyridine silver), and Lhamon *et al.* (1938; cat).

The anatomical demonstration of sympathetic fibres in the hypoglossal nerve passing to the blood vessels of the tongue corroborates the physiological observations of Morat (1930), who established that stimulation of the hypoglossal nerve leads to vaso-constriction in the tongue, a phenomenon abolished by removal of the superior cervical ganglion. A parallel can thus be drawn between the vasomotor nerve supplies to the tongue and the extremities, in which the sympathetic fibres reach their destination by coursing with the motor and sensory nerves (Woollard, 1926; Woollard & Phillips, 1932).

The intertwinings between sympathetic fibres are suggestive of those occurring between nerve fibres in coelenterates (Schaefer, 1878; Woollard & Harpman, 1939), where they have been ascribed a synaptic significance.

The finding of thin myelin sheaths on the larger sympathetic fibres in the hypoglossal nerve is in accordance with the work of Billingsley & Ranson (1918), Heinbecker & Bishop (1931) and Cleveland (1932), all of whom found myelinated fibres in the post-ganglionic branches of the superior cervical ganglion.

The present observations on the tongue as well as a study of methylene blue and gold chloride preparations of the muscles of the face, mastication, limbs and intercostal spaces in rabbits and cats confirm the findings of Woollard (1927), Wilkinson (1930), Hinsey (1930) and others in denying the existence of the accessory sympathetic innervation to striated muscles described by Boeke (1927), Agduhr (1919) and others. Woollard included in his studies the intrinsic musculature of the tongue of rats, cats and rabbits stained with methylene blue and gold chloride. The present investigations have also failed to disclose the accessory epilemmal motor plaques believed by Boeke (1913) and Langworthy (1924*b*) to be the endings of fibres contained in the chorda tympani nerve.

A fine network of argyrophil fibres has been described by Boeke (1933–6), Stoehr (1934–5), Reiser (1932, 1933) and Sunder-Plassmann (1930–5) in every tissue of the body and is believed by them to be part of the sympathetic nervous system. The nervous nature of this tissue, however, cannot be con-

sidered to have been established, since it has not been shown to disappear on complete denervation. Nonidez (1936, 1937) has shown in silver preparations that the fibres of the network in question are continuous with collagenous fibres, and considers them to be reticular connective tissue. This interpretation is in agreement with the present observations. The authors who describe so-called networks of nervous origin demonstrate their presence by the use of the Bielschowsky silver method, which does not select between nerve fibres and reticular connective tissue (Beech & Davenport, 1933).

Tarkhan (1936*b*) obtained in chloralosed cats a rise in blood pressure on faradization or pinching of the hypoglossal nerve. Acheson *et al.* (1936) recorded in urethanized cats a rise in blood pressure and slight contraction of the nictitating membrane on faradic stimulation of the central end of the cut hypoglossal nerve. Downman (1939) observed dilatation of the pupil in chloralosed cats and dilatation of the pupil, rise in blood pressure and muscular movements in decerebrate cats on pinching and on faradic stimulation of the hypoglossal nerve; the reactions could still be obtained after removal of the superior cervical ganglion. These experiments have been adduced as evidence for the existence of afferent fibres in the hypoglossal nerve. Dilatation of the pupil on stimulation of afferent somatic and autonomic nerves may be due to central inhibition of the oculomotor nucleus (Bain *et al.* 1934, 1935), or the impulse may reach the eye by travelling in the cervical sympathetic chain (Luchsinger, 1880; Anderson, 1904). The experiments on the hypoglossal nerve in rats recorded in the present paper suggest that the impulses reached the pupil via the cervical sympathetic system. The mechanism in these experiments might be explained by the occurrence of local reflexes such as Schwartz (1934) has demonstrated in the autonomic nervous system. Dilatation of the pupil, contraction of the nictitating membrane or rise in blood pressure as indices of afferent impulses must be accepted with caution. Acheson and his collaborators obtained contraction of the nictitating membrane and a rise in blood pressure on faradic stimulation of the hypoglossal, saphenous, "hamstring", splanchnic, hypogastric, hepatic, cervical sympathetic, pelvic and cardiac sympathetic nerves, but faradic stimulation of the cornea, while eliciting a rise in blood pressure, failed to produce contraction of the nictitating membrane. Langworthy (1924*b*) found no Sherrington "pain reaction" on faradization of the hypoglossal nerve in decerebrate cats. Barron (1936), in cats, rats and rabbits either decerebrate or under urethane or sodium amytal anaesthesia, was unable to record action potentials from the hypoglossal nerve on stretching, deforming or burning the tongue. Chemical destruction also gave negative results. Corbin & Harrison (1938), working with cats under pentobarbital sodium anaesthesia, failed to record action potentials indicative of proprioceptive impulses from the hypoglossal, lingual or upper cervical nerves on stretching or twisting the tongue. Downman obtained no dilatation of the pupil or rise in blood pressure in cats in which the chorda tympani, lingual and glossopharyngeal nerves had been cut on pinching, crushing or

burning the tongue, nor on stretching to the extent of tearing the muscle fibres. The author believes that the failure to obtain reflex responses in these experiments can be accounted for by the inadequacy of the stimuli employed. Such an interpretation is not in accordance with physiological experience, since reflex responses and action currents can be elicited by gentle pressure on, or stretching of, muscles known to contain sensory nerve endings.

Carleton (1937) painted the mucosa of the tongue and buccal cavity with 5–10 % cocaine in eight subjects. In four subjects the only sensation that could be aroused was one of traction at the base of the tongue. In the remaining four individuals the sense of position did not wholly disappear, possibly because of incomplete loss of tactile sensation, due, the author suggests, to inadequate anaesthetization. The results of Carleton's as well as the present series of clinical observations can be explained by the presence of sensory endings at the origin of the genioglossus muscle.

The sensory overlap found in the region of the lower gum is in accordance with similar clinical findings of Stewart & Wilson (1928). The overlap is explained by the finding of anastomotic fibres at the symphysis in dissections of human material (Starkie & Stewart, 1931). Sensory overlap in the midline has not been found anywhere else in the body.

Matthews (1933) infers from action-potential studies that neuro-muscular spindles, by virtue of their "flower spray" endings, are stimulated by passive stretch of the muscle, and cease to discharge during active contraction of either the surrounding or the intrafusal muscle fibres. The "annulo-spiral" endings are considered to act similarly except that when the intrafusal muscle fibres contract the rate of discharge increases. There would thus appear to be no necessity for structures endowed with "flower spray" endings in muscles that have no postural antagonists. This interpretation is in accordance with the fact that no neuro-muscular spindles can be demonstrated in the striated musculature of the larynx, pharynx and oesophagus, the diaphragm, the bulbo- and ischio-cavernosus muscles (Sherrington, 1897; Schaffer, 1922) and the muscles of the face. The extrinsic muscles of the eye are apparently the exception to this generalization, since they are now generally considered to contain no neuro-muscular spindles. The "terminaisons en grappe" that have been described by Woollard (1927, 1937) and others in the extrinsic ocular muscles, however, are similar to "flower spray" endings and probably subserve the same function. The tongue also has no postural antagonists, and thus it is not surprising that it has no neuro-muscular spindles.

Matthews (1933) concludes that neuro-tendinous endings respond to either passive stretch or active contraction. They are presumed to be the tension recorders "par excellence" (Fulton, 1938). Sensory endings have been found at the insertion of the genioglossus muscle. They are similar to the "ball-like" nerve terminations described by Stoehr (1932*a*, *b*) in the pia mater and considered to record changes in tension. Although this type of ending differs morphologically from the neuro-tendinous endings of Golgi, it probably sub-

serves similar functions. The origin of the nerve fibres supplying the sensory endings at the origin of the genioglossus muscle has not been determined.

In the extrinsic muscles of the eye the functions of the "annulo-spiral" ending of neuro-muscular spindles may be subserved by neuro-tendinous organs which, though smaller (Sherrington, 1897), closely resemble the tendon nerve endings of Golgi.

SUMMARY

1. The innervation of the intrinsic and extrinsic musculature of the tongue has been studied histologically by a modified methylene blue technique in a series of thirty-seven normal and eleven operated rats and one rabbit in which the hypoglossal and lingual nerves and the cervical sympathetic chain had been resected alone or in various combinations.

2. The staining technique is based upon the injection of measured quantities of solutions of methylene blue in normal saline into tissues in which the circulation is maintained. The method described is applicable to the musculature of the tongue in rats and rabbits and the skin of the ear in the rabbit.

3. No sensory endings were found in the intrinsic muscles of the tongue.

4. No neuro-muscular spindles were found in the hyoglossus or genioglossus muscle.

5. Sensory endings of a tension-recording type were found at the origin of the genioglossus muscle.

6. No nerve cells were found along the course of the hypoglossal nerve in the tongue.

7. Sympathetic fibres run in the hypoglossal nerve to reach the blood vessels of the tongue.

8. No accessory nerve endings to muscle fibres or motor end-plates were found.

9. The sympathetic ground plexus, periterminal network and terminal reticulum are considered to be reticular connective tissue. No nerve nets were observed.

10. Dilatation of the pupil on stimulation of the hypoglossal nerve in rats under light ether anaesthesia was found to be due to stimulation of the sympathetic fibres in the hypoglossal nerve.

11. Proprioception, taste, pain and touch were tested on the tongue, lower gum and lip in nineteen human subjects in which the lingual and inferior alveolar nerves had been unilaterally or bilaterally infiltrated with novocain, or in which the mucosa of the tongue, oral pharynx and palate had been painted with "decicain" or cocaine.

12. In cases in which the lingual nerve was anaesthetized by infiltration the area of ageusia was limited to the anterior two-thirds of the tongue and there was no overlap in the midline.

13. Pain and touch were found to overlap in the midline in the region of the lower gum. This was confirmed in a case of central root division of the trigeminal nerve.

14. All forms of sensation tested in the anterior two-thirds of the tongue were found to be abolished on complete anaesthetization.

15. There was no ataxia or tendency to bite the tongue after complete anaesthetization, although articulation was difficult.

16. The mechanism of proprioception is discussed in the light of the anatomical and clinical findings and recent physiological work.

The authors wish to thank Mr J. L. Dudley Buxton for the facilities accorded at the National Dental Hospital. Thanks are also due to Miss Z. Stead for help with the experimental procedures, Mr F. J. Pittock for the preparation of the photomicrographs and Miss I. Sachs for skilful assistance.

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EXPLANATIONS OF PLATES I–IV

All the figures are photomicrographs from methylene blue preparations.

PLATE I

Fig. 1. Rat. Normal. Intrinsic musculature of tongue. Thick motor nerve fibres; thin autonomic nerve fibres. ×80.

Fig. 2. Rat. Normal. Intrinsic musculature of tongue. Sympathetic fibre (arrows) emerging from a fasciculus of the hypoglossal nerve. The fibre becomes gradually thinner and eventually ends on a blood vessel. ×430.

PLATE II

Fig. 3. Rat. Hypoglossal nerve sectioned at base of skull prior to its junction with branches from the superior cervical ganglion. Intrinsic musculature of tongue. Termination on a capillary of a sympathetic fibre conveyed by the hypoglossal nerve. ×530.

Fig. 4. Rat. Normal. Intrinsic musculature of tongue. Motor end-plate. There are no accessory fibres. ×530.

Fig. 5. Rat. Hypoglossal nerve cut at base of skull proximal to its junction with branches from the superior cervical ganglion. Three sympathetic fibres among degenerated motor fibres in a fasciculus of the hypoglossal nerve in the tongue. ×580.

Fig. 6. Rat. Hypoglossal nerve cut at base of skull proximal to its junction with branches from the superior cervical ganglion. Intrinsic musculature of tongue. An intertwining between two varicose sympathetic fibres. ×725.

Fig. 1.

Fig. 2.

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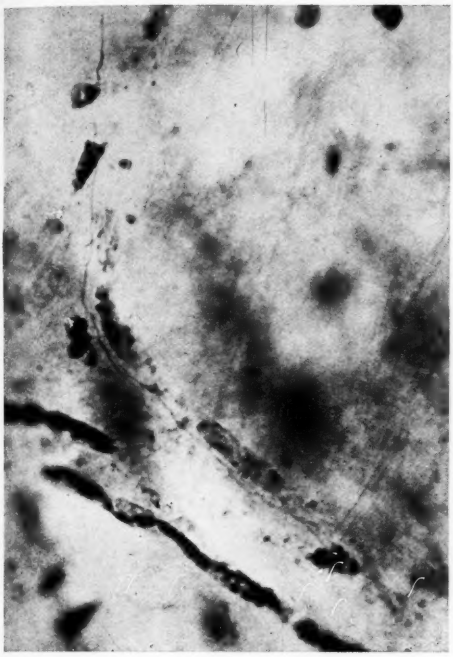
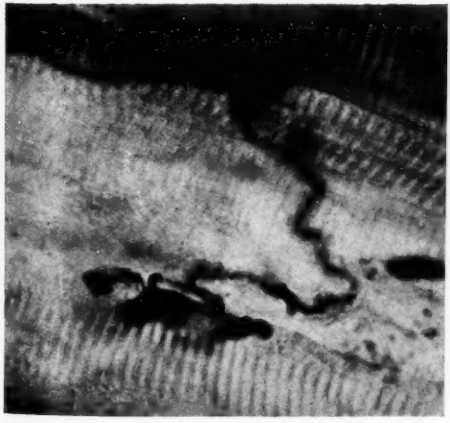


Fig. 3.

Fig. 5.



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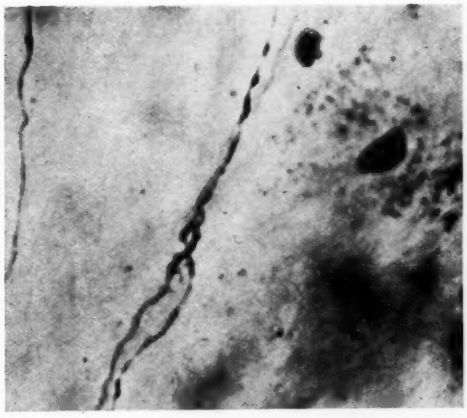

Fig. 6.

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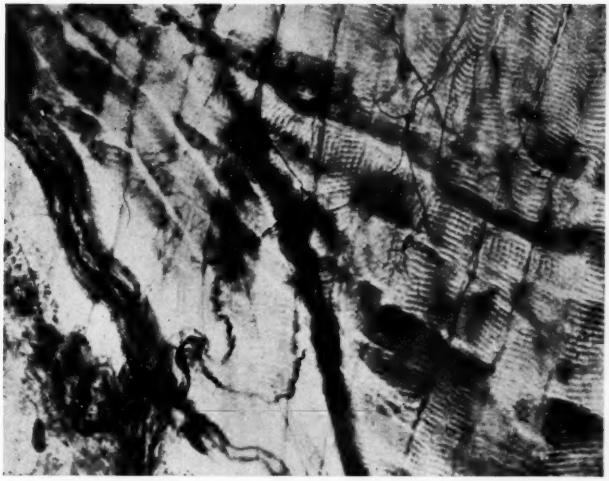

Fig. 7.

Fig. 8.

Fig. 9.

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Fig. 10.

Fig. 11.

Fig. 12.

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PLATE III

Fig. 7. Rat. Normal. Intrinsic musculature of tongue. Reticular connective tissue fibres. ×250.

Fig. 8. Rat. Normal. Region of attachment of genioglossus muscle to the mandible. A sensory ending of tension recording type. ×120.

Fig. 9. Rabbit. Leg. Extensor digitorum longus muscle. Annulo-spiral ending of a neuro-muscular spindle. ×440.

PLATE IV

Fig. 10. Rat. Hypoglossal nerve cut at base of skull proximal to its junction with branches from the cervical ganglion. Intrinsic musculature of tongue. Stained circular muscle fibres around an arteriole and nerves supplying the vessel. ×440.

Fig. 11. Rabbit. Leg. Extensor digitorum longus muscle. Neuro-muscular spindle showing the characteristic innervation, shape, length and type of muscle fibre. ×220.

Fig. 12. The annulo-spiral ending of the neuro-muscular spindle illustrated in Fig. 11 seen in more detail. ×440.

THE DEVELOPMENT OF NERVE-ENDINGS IN THE RESPIRATORY MUSCLES OF THE SHEEP

By LILIAN M. DICKSON

Department of Anatomy, London School of Medicine for Women

The recent work of Barcroft *et al.* (1936), and of Barcroft & Barron (1936, 1937) on the genesis of somatic movement in sheep foetuses included a study of the development of respiratory movement, and by the kindness of Sir Joseph Barcroft the histological examination of respiratory structures was entrusted to the author. The present paper deals with the condition of nerves and nerve-endings in the intercostal muscles and diaphragm of some of the subjects of the Cambridge experiments, and attention has been specially directed to the development of muscle spindles and motor end-plates.

The first object of the investigation was to show what peripheral nervous structures are present in respiratory muscles when respiratory movement is developing, and if possible to throw some light on their function. But the examination of a series of foetal material has also made possible a study of the course of development of muscle spindles and motor end-plates, and has established for one vertebrate species within fairly narrow limits the age at which these structures begin to form.

MATERIALS AND METHODS

The material used was provided by Sir Joseph Barcroft from foetuses whose movements had previously been studied by himself and Dr Barron. It consists of the respiratory muscles of twelve sheep foetuses of ages ranging from 40 to 139 days of gestation.

The tissues of nine of the twelve foetuses were fixed in 7% formol and impregnated by Hewer's (1933) modification of Ranson's silver method. In addition, Bodian's (1936) silver method was used for some of the material from two of the nine foetuses. Paraffin sections were cut serially at 7·5 and 10 μ.

The Ranson sections for the most part show good impregnation; Bodian's method reveals very clearly the cross-striation of the muscle fibres and the axis cylinders but has not picked out nerve-endings, probably because alcohol fixation, which Bodian advises, was not possible in this case. Only those pieces of material which showed good preservation and impregnation by silver were used; and for each foetus at least 100 sections each of diaphragm and intercostal muscle were examined, and in most cases more than double that number. Hence, while recognizing the erratic character of silver impregnation, it is felt that a fair measure of reliance can be placed on negative as well as positive findings in respect of nerve-endings in these specimens.

The remaining three foetuses were prepared in the Cambridge laboratories. They were stained by de Castro's silver method and show excellent impregnation.

Table I. *Number and age of specimens.* (*Gestation period of sheep* = 157 *days*)

No.	Age (days)	Method of silver impregnation
1	40 (foetal)	de Castro
2	45	,,
3	47	Ranson (Hewer's modification)
4	58	de Castro
5	66	Ranson (Hewer's modification)
6	80	,, ,,
7	84	,, ,,
8	92	,, ,, and Bodian
9	96	,, ,,
10	100	,, ,, and Bodian
11	111	,, ,,
12	137	,, ,,

HISTOLOGICAL FINDINGS

The muscle fibre

(*a*) *Intercostal muscles.*

In foetuses 1–3 (40–47 days), muscle fibres are very slender, faint cross-striation is visible, and centrally placed nuclei form swellings on the course of the fibres. Fibrils are faintly visible.

In foetus 5 (66 days) the short oval nuclei just fill the width of the fibre.

In foetus 6 (80 days) the nuclei begin to take up a lateral position and cross-striation is well defined.

In foetus 12 (137 days) the muscle fibre has all the adult characters.

(*b*) *Diaphragm.*

In foetuses 1–5, the muscle fibres appear slightly less mature than those of the intercostal muscles of the same foetuses; but in the 80-day and older specimens there is no noticeable difference between the fibres in the two groups of muscles.

Nerve fibres and nerve-endings

(*a*) *In intercostal muscles.*

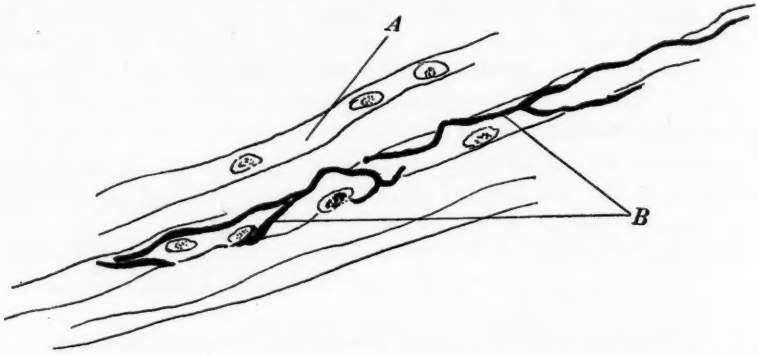
Undifferentiated endings. In foetus 1 (40 days) nerve fibres are plentiful, and many terminate in contact with muscle fibres, but no differentiated endings are present. It seems reasonable to presume that some of these endings are motor, although it is not possible to assign a function with any certainty (Pl. II, fig. 1).

Sensory endings. In foetuses 2 and 3 (45 and 47 days) formations suggesting very early spindles are present. Since a good photograph of these structures was unobtainable, a camera lucida drawing of one of them, found in the 45-day specimen, is given (Text-fig. 1).

Fine branches of a nerve fibre partially enwrap one or more muscle fibres, forming a very simple network. The structure of the muscle fibre was not clearly made out. There is no fusiform enlargement, but the whole fibre is thicker than those in the vicinity, and it may be that more than one is enclosed within the nervous network. There is no surrounding connective tissue reaction.

In foetus 4 (58 days), endings were found similar to those just described but more clearly defined, and a sensory function can be assigned to them with some confidence. One is photographed (Pl. I, fig. 1); as in the younger specimen, there is no fusiform enlargement of the muscle fibres, no surrounding capsule and no lymphatic space.

In foetus 5 (66 days) a clearly defined spindle (Pl. I, fig. 2) is present. It lies within a lymphatic space and consists of a coarse nervous network enclosing several fine muscle fibres. It is not yet "spindle" shaped. In all



Text-fig. 1. Camera lucida drawing of an early sensory formation, probably a muscle spindle, in the intercostal muscle of a 45-day sheep foetus. × 380. A, muscle fibre. B, sensory formation (the thickness of the component branches is slightly exaggerated).

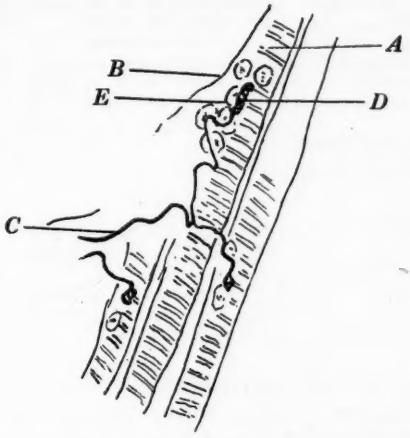
the older foetuses, spindles were found, and by mid-term they are well formed (Pl. I, fig. 3). In the 90-day specimen spindles are long, fusiform, and highly complex in structure. Free interfascicular endings were not found in abundance but are certainly present at 80 days. One is seen in Pl. II, fig. 2. In the absence of any neighbouring blood vessel, its sensory rather than autonomic character may be safely assumed.

Motor endings. As stated above, at 40 days many nerve fibres end in contact with muscle fibres but they do not differ from one another, and so it is impossible to assign to them their different functions.

With the beginning of spindle differentiation at 45 days, it can safely be assumed that many of the still undifferentiated endings are motor, but they undergo surprisingly little change even up to 100 days. The fibre may end singly, or by simple branching, or in a spray, and there are tiny beads at each terminal twig. In the older specimens (80–100 days), the fibres thicken, the

beads slightly enlarge and the fibre tends to approach the muscle fibre at right angles to its long axis. This mode of approach is perhaps the most characteristic feature of the motor fibres.

In the 111-day foetus the motor ending is better defined. The terminal "bead" takes the form of an oval plate in which a network is apparent. In one specimen observed, the axis cylinder appeared to branch into two just before reaching the muscle fibre and both branches ended in oval swellings on the same muscle fibre. This seems to be the stage just before true "motor plate" formation. There is as yet no sign of nuclear aggregation into a sole plate (Pl. II, fig. 3).



Text-fig. 2. Camera lucida drawing of a young motor end-plate in the intercostal muscle of a 137-day sheep foetus. ×380. (A microphotograph of the same structure appears on Pl. II, fig. 4.) A, muscle fibre with B, sarcolemma torn apparently near junction with neurilemma. C, nerve fibre. D, motor end-plate. E, sole plate.

In the 137-day foetus a true motor plate is present and is figured (Pl. II, fig. 4 and Text-fig. 2). It is far from fully differentiated, but the "sole" has formed and the ending is clearly hypolemmal in position.

In the younger specimens, the relation of the ending to the sarcolemma could not be established with certainty.

(*b*) *In diaphragm.*

Sensory and motor endings. In the younger specimens development runs much the same course as in the intercostal muscles with perhaps a slight lag. Foetus 6 (80 days) was the youngest in which a spindle was found but it is difficult to believe that they are not present earlier. At 84 days, fairly complex spindles are present. A 90-day specimen has been photographed (Pl. I, fig. 4). As in the intercostal muscles, a true motor end-plate is seen only in foetus 12 (137 days) and is then still in an early stage of development.

Table II. *Motor and sensory nerve-endings in intercostal muscles and diaphragm of foetal sheep (gestation period = 157 days)*

Foetus no.	Age in days	Intercostal muscle		Diaphragm sensory and motor
		Sensory	Motor	
1	40	Nerve fibres in contact with muscle fibres. Endings not differentiated		Muscle not fully formed No endings observed
2	45	Formations suggesting early spindle	Simple beaded endings, not clearly differentiated	Motor as in intercostal
3	47			No sensory formations seen
4	58	Better defined "pre-spindle"		No material
5	66	Simple, but definite spindle	Endings numerous, single, spray-like, or show simple branching	Motor as in intercostal, no spindles observed
6	80	Free interfascicular ending formed	Fibres tend to approach muscle fibres at right angles to long axis of muscle	Well formed spindle
7	84	Well-formed spindle	,,	As in intercostal
8	92	Complex spindle	,,	,,
9	96	,,	,,	,,
10	100	,,	,,	,,
11	111	,,	Fibres now end in oval swellings of plexiform structure	,,
12	137	,,	True end-plate, of simple form, with well-defined sole plate	,,

The results may be briefly summarized as follows:

Appearances suggestive of early spindle formation are present at 45–47 days; spindles are well defined, though simple at 66 days and well formed by 84 days. Motor end-plates are not present at 111 days; they are present in simple form with true sole plate at 137 days.

DISCUSSION AND CONCLUSIONS

(1) *The present findings compared with the results of other workers*

The finding of well-developed muscle spindles at an age at which the earliest differentiation of motor nerve-endings is hardly discernible is in accordance with the results of other workers in this field. Tello (1917, 1922) finds well-marked muscle spindles in the cat embryo at 30 days and in the human foetus at 6 months, whereas in the 30-day kitten motor end-plates have not begun to form and in the human foetus at term they are still incomplete, although in some muscles, for example the tongue, they approach completion. Hewer (1935), working on human material, finds "fairly complete" spindles at 20 (foetal) weeks when the motor nerve-endings are just beginning to differentiate, and makes the further observation that in the human foetus at term the motor nerve-endings in the tongue, diaphragm and intercostal muscles are fully formed, although they are still immature in the

muscles of the limbs. Hewer has also examined the neck muscles of some of the foetuses used for the present investigation and obtained the following results which she allows to be quoted, namely that at 80 days primitive spindles are present, but at 137 days there is still no sign of developing motor end-plates. Table III shows the comparative results.

Table III. *Age of appearance of nerve-endings in different vertebrate species*

Animal	Region	Foetal age days	Results	Gestation period days
		A. Muscle spindles		
Sheep	Respiratory muscles (author)	45	Suggestive sensory formation	157
	,, ,,	66	Definite spindle	
	,, ,,	80	Well-formed spindle	
	Neck muscles (Hewer)	80	Primitive spindle	
Chick	? (Tello)	14	Well-formed spindle	21
Cat	Foot muscles (Hewer)	30	,, ,,	60
Human	Limb muscles (Hewer)	140	,, ,,	280
	Leg muscles (Tello)	168	Very complex spindle	
		B. Motor endings		
Sheep	Respiratory muscles (author)	137	Simple motor end-plate with sole plate	157
	Neck muscles (Hewer)	137	Not begun to form	157
Human	Tongue (Hewer)	200	Motor plates present but unfinished	280
	,, ,,	280	Highly differentiated	280
	,, (Tello)	280	Fairly well-developed with sole plate	280
	Intercostals (Hewer)	280	Highly differentiated	280
	,, (Tello)	280	Slightly less than tongue	280
	Forearm (Hewer)	200	Just beginning to differentiate	280
	Leg (Hewer)	280	Not nearly completed	280
	Leg (M. soleus) (Tello)	280	Beginning	280

These comparisons bring out two points of interest.

(1) In the species examined muscle spindles are present and generally well formed by mid-term, whereas motor end-plates only begin to differentiate after mid-term.

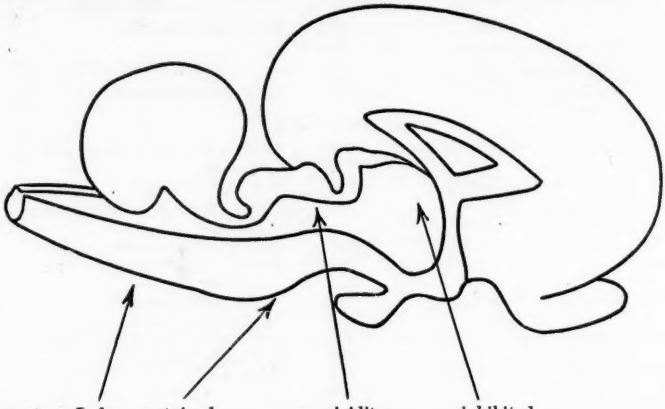
(2) In the sheep at least, both muscle spindles and motor end-plates develop earlier in intercostal and lingual muscles than in the muscles of the limbs, and the early development of motor end-plates in the human tongue and intercostal muscles suggests that a similar age difference may exist in other species. Whether this difference has a functional significance cannot at present be discussed for lack of sufficient data.

(2) *Relation of the age of appearance of nerve-endings to length of gestation period and the maturity of the species at term*

In comparing the age of development of nerve-endings in different species it is convenient to correlate the histological findings with the length of the gestation period, but in using these results to assess the probable time of appearance of nerve-endings in another species, it may be necessary to take

into account a further factor, namely the degree of maturity attained by the animal at term. Nerve-endings which are present at mid-term in the sheep, which is post-mature at birth (as compared with the human baby), might well not appear until much later in the rat, for example, which is very immature at birth. In fact, if the findings recorded above in respect of sheep are representative of a wide range of animals, one would not expect to find completed motor end-plates in the rat until well after birth. It is hoped to obtain exact evidence on this point shortly.

The matter is of some interest in view of the vast body of contemporary experimental work, some of it on foetal material, dealing with the release of transmittor substances at the neuro-muscular junction. The presence or absence of a sole plate, amongst other histological factors, might well affect chemical transmission.


Movement
Jerky→sustained→rigidity→inhibited
Respiration
gasping→continuous→inhibited
Age in days
40 45 50 55 60 65 70 75 80 85
Condition of nerves and nerve-endings in respiratory muscles
Nerve fibre in contact with muscle fibre.
Suggestive "pre-spindle" formation (45)
Well-defined "pre-spindle" (58)
Definite simple spindle
Well-formed spindle
No endings differentiated
No motor end-plates. Motor fibres end simply or in simple branching or sprays with small terminal beads

Text-fig. 3. This record of observed movements was made by Dr D. H. Barron, and is reproduced by his permission. The histological findings were added by the author.

(3) *Relation of muscle-spindles and motor end-plates to respiratory movements*

The results of Barcroft & Barron's extensive studies of the genesis of somatic movement in sheep foetuses have been published (1936*b*, 1937), and the reader is referred to those papers. One set of observations will be considered here. They are summarized in the accompanying chart (Text-fig. 3)

made by Dr D. H. Barron, and published here by his permission, with the addition of the author's histological findings. Barcroft & Barron found that movement at different ages shows certain well-defined characters. At first jerky, it gradually becomes sustained, and in older specimens the phenomena, first of rigidity and later of inhibition, are observed. Sustained movement is established by the 48th day. Further, it was found that these changes in muscular behaviour are associated with the forward extension of pathways and the development of inhibition centres in the brain stem (diagram, Text-fig. 3). These observations refer to movements as a whole, including those of the trunk, head and limbs. Coming to the special case of respiratory movements the further observations were made:

(1) The earliest involvement of the respiratory musculature was observed in a 38-day foetus and was part of a mass movement of the body. The separation of respiratory from general body movement develops gradually between the 38th and 49th days by the dropping out of the movements of the head and limbs.

(2) The respiratory movement is a rhythmical one. The rhythm is at first jerky and of short duration; it gradually becomes more sustained; and by the 50th day respiratory movements are almost continuous and are of the sustained or tonic type (1937).

(3) The development of a tonic element in the rythmic movement depends on the development of central inhibitory mechanisms (1937).

Turning to the histological findings, we find that:

(1) At 40 days, i.e. 2 days after the earliest exhibition of respiratory movement, no specialized nerve-endings are present.

(2) By 50 days, when the change from "jerk" to sustained movement is fairly well established, a specialized sensory ending, the "pre-spindle" of 45 days, has made its appearance.

(3) The establishment of sustained movement and the manifestation of inhibition take place long before the earliest development of a motor end-plate.

Rhythmic respiratory discharges from the central nervous system depend upon the excitability of the brain which is heightened by the arrival of sensory stimuli—indeed it is an open question whether without such sensory stimuli the excitability would not be below the linal level. One of the obvious sensory avenues is from the nerve endings in the respiratory muscles themselves. The histological findings show that undifferentiated endings, possibly in contact with the muscle fibre, are able to subserve jerky rhythm. Whether for sustained rhythm a more complex type of receptor organ is essential cannot be said with certainty from the available data, but it is at least suggestive that the beginning of spindle formation and the development of sustained movement should so closely coincide.

SUMMARY

1. The muscle fibre and nerve-endings in the intercostal muscles and diaphragm of 12 sheep foetuses are described.

2. The formation of the muscle spindle is found to begin at about the 45th day of gestation and to be well advanced by mid-term (about 80 days).

3. Motor end-plates are not found at 111 days and are present in simple form at 137 days.

4. The findings (i) are compared with those of other workers in this field, and (ii) are considered in relation to observed movements.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Sir Joseph Barcroft for providing the material on which my results are based.

My thanks are also due to Dr D. H. Barron for allowing me to use his diagram and chart, to Dr E. E. Hewer for communicating her unpublished findings, and to Prof. M. F. Lucas Keene for her helpful criticism and advice at many stages of the work.

The micro-photographs were made by Mr Willmott of the British Post-graduate School.

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EXPLANATION OF PLATES I AND II

Plate I

Figs. 1–4. Microphotographs of developing muscle spindles in the respiratory muscles of sheep foetuses.

Fig. 1. 58-day foetus, intercostal muscle. ×750.

Fig. 2. 66-day foetus, intercostal muscle. ×450.

Fig. 3. 84-day foetus, intercostal muscle. ×450.

Fig. 4. 90-day foetus, diaphragm. ×300.

Plate II

Figs. 1–4. Microphotographs of developing nerve-endings in intercostal muscles of sheep foetuses.

Fig. 1. Undifferentiated nerve-ending in a 45-day foetus. ×335.

Fig. 2. Free interfascicular sensory ending in an 80-day foetus. ×335.

Fig. 3. Motor ending in a 111-day foetus. ×750.

Fig. 4. Young motor end-plate with sole plate (note direction of arrow) in a 137-day foetus. ×450.

Fig. 1.

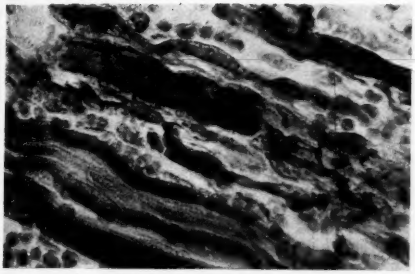

Fig. 2.

Fig. 3.

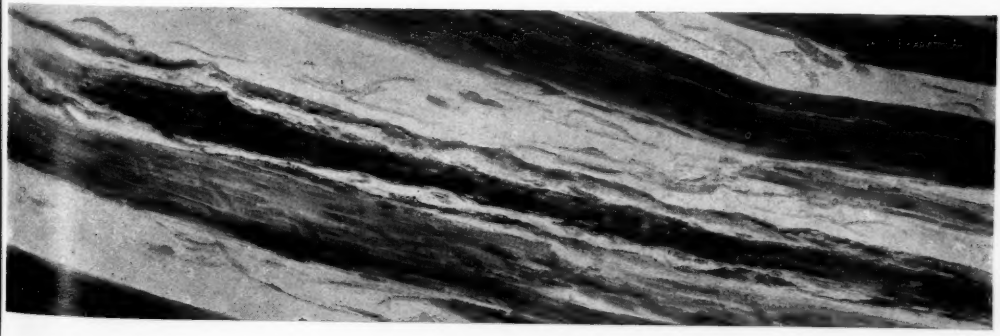

Fig. 4.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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THE DEVELOPMENT OF SYNOVIAL JOINTS

By JAMES WHILLIS

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Most of the descriptions of the development of synovial joints give the impression that the synovial cavity is formed by a process of absorption. It is the purpose of this paper to review the factors concerned in the formation and extension of the synovial cavity as seen in the development of the human embryo and the white rat.

Observations have been made on the interphalangeal joints of the foot in a series of human embryos varying in length from 30 to 125 mm. Much material of this kind available had to be rejected because of shrinkage due to inadequate fixation and this led to a supplementary study of rat embryos, where the age could be determined with accuracy and fixation adequately controlled.

For the rat embryos used in the investigation I am indebted to Mr T. McKeown of Guy's Hospital.

The human material was fixed in formalin and the rat limbs were fixed in Bouin's solution. After fixation and clearing, the tissues were first infiltrated with wax of 45° melting point at a temperature of 50° C. and then transferred to 60° wax at 62° C. for a further period of 1 hour.

The appearances seen in the human specimens will be described first and their significance discussed later.

30 MM. STAGE (Fig. 1)

A section of one of the interphalangeal joints at this stage shows the two chondrifying elements separated by relatively undifferentiated mesenchymal cells constituting the "joint disk". The cells of the disk are arranged in arcs parallel with the surfaces of the cartilage elements. In that part of the disk in the region of the future articular surfaces the cells are flattened, very closely packed and disposed with their long axes in the joint line. At the extreme periphery of the disk the mesenchymal cells are disposed longitudinally and here the mesenchyme is continuous with the condensation forming the perichondrium. In the peripheral part of the disk, where there is a change from the arc to the longitudinal arrangement of the cells, the mesenchyme is much looser than in the central part of the disk where the cells are very closely packed.

45 MM. STAGE (Fig. 2)

The cartilage elements are developing their shape, a convex surface proximally, and a concave surface distally. The joint disk of mesenchyme is reduced in thickness and the loose arrangement of the cells at the periphery is still more

evident, and cavities are appearing among them. The central part of the disk is formed of flattened closely packed cells with their long axes parallel with the future joint surfaces.

63 MM. STAGE (Fig. 3)

Further reduction of the thickness of the joint disk has occurred and it is now represented by a layer two cells in thickness between the two cartilage elements. The synovial cavity is present in the circumferential part and, in serial sections, can be traced all round the joint. The mesenchymal cells nearby are becoming arranged parallel with the boundaries of the synovial cavity.

72 MM. STAGE (Fig. 4)

The joint disk is represented by a single layer of flattened cells. The circumferentially placed synovial cavity is well defined and lined with flattened cells. On the left of the section, the mesenchyme is forming a rudimentary meniscus in continuity laterally with the lining of the synovial cavity and medially with the joint disk. The shape of the future articular surfaces is well defined.

125 MM. STAGE (Fig. 5)

The joint disk between the cartilages has entirely disappeared and the two elements are united across the joint line by primitive cartilage, or "precartilage". The matrix of this primitive cartilage has a staining reaction rather like that of collagen with haematoxylin and eosin, but with thionin, although not so dark as the more mature matrix, it gives the typical reaction of cartilage. Near the margin of the area of union the matrix shows little affinity for stains and is apparently undergoing liquefaction. There is, at this stage, no extension of the circumferential synovial cavity between the two cartilage elements. On the right of the section the meniscus formed by the remains of the original mesenchymal disk is free of the cartilage elements; on the left, the meniscus is attached to the surface of the proximal cartilage and contains some young chondroblasts. Menisci of this type are found at this stage in nearly all the joints examined.

DISCUSSION

It is convenient first to review the development of joints up to the point where the elements are united across the joint line by primitive cartilage and the joint cavity is limited to the circumferential region, and to consider later the solution of this bond and the extension of the cavity between the two cartilages.

The work of Fell (1925) has shown that at an early stage the mesenchymal core in the limb bud is determined in respect of the pattern of future chondrification in the process of laying down of the skeletal elements. Experiments by Fell & Canti (1934) show, moreover, that the early stages of joint formation described above take place in almost identical fashion in the avian knee joint

cultivated *in vitro*. There is progressive diminution in the thickness of the mesenchymal disk between the developing cartilages, loosening of the mesenchyme in the circumferential part of the disk, and finally union of the two skeletal elements by primitive cartilage. These experiments show conclusively that the mesenchyme of the joint disk does chondrify, and dispose of the theory that the joint is formed because of the interposition of non-chondrifying tissue between the two bones. Carey (1922) described the tissue uniting the cartilages as "mucoid", but Fell's experiments and observations on normal embryonic joints have demonstrated that it is very young cartilage.

Recent experiments by Glücksmann (1938) have shown that undifferentiated mesenchyme subjected to pressure between two developing cartilages itself undergoes chondrification. The progressive chondrification of the joint disk occurring in normal development is, therefore, consistent with the disk mesenchyme being subjected to pressure. The fact that there are many mitotic figures in the cells of the cartilage in the immediate vicinity of the disk during this period of development probably indicates that the progressive diminution in the thickness of the disk is due partly to the conversion of mesenchymal cells into chondroblasts and partly to the invasion of the disk by actively multiplying chondroblasts from the ends of the cartilages.

To postulate pressure on the mesenchymal disk it must be assumed that the cartilage elements are growing very much faster than the sleeve of tissues surrounding them in the limb bud. The assumption is reasonable because the cartilage is growing not only by multiplication of its cells but also by the addition of a relatively large amount of intercellular substance.

That the progressive chondrification of the joint disk is due in part to the effect of pressure is, however, supported by evidence available when the disk mesenchyme is protected from such pressure. The region of the wrist affords the first example of this.

A coronal section of the wrist region of a human embryo of 30 mm. is shown in Fig. 6. The early stages of joint formation are evident between all the cartilage elements. The cartilage of the ulnar styloid is long and curved, and is separated from the triquetral cartilage by a well-marked joint disk which resembles in every way those seen where synovial joints will later be formed. The presence of the early stages of joint formation between the ulnar styloid and the triquetral in the human embryo is of special interest because in the macaque monkey a large synovial joint is present in this region (Wood Jones, 1920). It will be obvious from reference to Fig. 6 that the mesenchyme between the *head* of the ulna and the triquetral will be protected from counterpressure exerted by the growing cartilages because of the apposition of the ulnar styloid to the triquetral. Fig. 7 shows the appearance at the 125 mm. stage. The styloid process of the ulna is much smaller relative to the size of the head than it is at the 30 mm. stage. It is separated from the triquetral by a considerable interval and is not so curved as in the earlier stage of its formation. A comparison between Figs. 6 and 7, moreover, shows that the growth of the

radius has apparently pushed the carpal elements distally away from the ulna which seems to be growing rather more slowly than the radius. Between the individual carpal elements, and between the lower end of the radius and the carpus, the joint disks have chondrified and the elements are united across the joint lines by primitive cartilage. Between the head of the ulna and the triquetral, however, where the previous apposition of the ulnar styloid to the carpus has protected the mesenchyme from pressure, it has not chondrified but has become modified to form the articular disk of the inferior radio-ulnar joint. Where the withdrawal of the ulnar styloid from the triquetral has resulted in relief of pressure on the joint disk originally present between the two elements, the disk mesenchyme has not chondrified but has become stretched and incorporated in the medial ligament of the wrist joint.

The features seen at the two ends of the developing clavicle afford similar evidence. The appearance, very early in development, of the centres of ossification in the clavicle leads to a slowing of the growth of the precartilage of which it is formed at a period when the mesenchyme at the two ends is relatively undifferentiated. The relief of pressure on this mesenchyme removes the stimulus for its further chondrification and it becomes modified to form the articular disks of the sterno-clavicular and acromio-clavicular joints.

If there be pressure on the joint disk in the earlier stages of development, such pressure would assist in the formation of the circumferential synovial cavity. Fig. 8 shows a disk formed of laminae of sheet rubber subjected to pressure by a clamp. Compression of the central part leads to a reduction in thickness, whilst in the part not subjected to pressure the sheets tend to separate. The orientation of the mesenchymal cells in the joint disk in Fig. 3 bears a striking resemblance to the arrangement of the rubber sheets in the model.

The way in which the solution of the temporary continuity between the bone elements is brought about must now be considered.

The fact that the union of the two skeletal elements by primitive cartilage persists in the human embryo up to the fourth or fifth month, and is not observed after that period, makes it tempting to assume that foetal movements are the determining factor in the completion of the process of joint formation. The evidence that this is so is, however, scanty and unconvincing. In the first place, the facts might just as well be explained by assuming that movement does not occur because joint formation is not complete as by assuming that it is movement that completes the formation of the joint. Moreover, there is very definite evidence that movement cannot be the sole factor in the extension of the joint cavity between the bone elements. Hamburger (1928) has demonstrated the formation of normal joints in the hindlimbs of frogs in which the muscles had been paralysed by denervation. This observation in itself does not, however, exclude movement completely as a factor in joint formation. First, Hamburger describes, in two cases, complete cartilaginous union between the femur and the hip bone and similar fusion in the tarsal joints in a few cases.

Secondly, the possibility of passive movements cannot be excluded in these experiments. A description by Nicholson (1937) of the formation of perfect synovial joints in a hand-like appendage occurring in a teratoma does, however, show quite definitely that normal joints can develop in the complete absence of movement. In the appendage, very carefully examined and described by him, no trace of muscle could be found. Experiments by Straus (1938) on the occurrence of movements in rat embryos on stimulation over the region of the brachial plexus showed that movement could be elicited in the proximal joints of the upper limb at the 14 mm. stage but not in the region of the carpus or digits. This conforms with the fact that the differentiation of the joints proceeds proximo-distally but, as the movements occurred under abnormal stimulation, it cannot be taken as evidence that, in normal conditions, even the proximal joints were really capable of movement. Indeed, the section of the elbow joint shown in Fig. 9 demonstrates union of the humerus and the ulna by primitive cartilage after birth, and no movement of the elbow region was detected in the rat from which the section was taken. Straus's experiments do show that in conditions of abnormal stimulation the muscles, which are capable of contracting, may break the bond of union so that movements can take place at a time when they do not normally occur. This therefore suggests that movement, although not the sole factor, may be one of the factors in normal joint formation. In the tissue culture experiments conducted by Fell and Canti passive movements of the two cartilage elements failed in every case to prevent their subsequent cartilaginous fusion. This confirms the evidence that some additional factor is essential in the formation of the joint.

In the search for other factors which may be concerned it is interesting to note that it is only in tissue explants cultivated *in vitro* that the cartilaginous union between the two bone elements persists in all cases. In the other examples which have been considered, viz., normal development, denervated limbs, and the teratoma, there is solution of this continuity and the formation of the completed synovial joint. A tabulation of the conditions present in the cases under review is given below:

	Nerves	Vessels	Muscles	Calcification	Persistent union
Embryo	Yes	Yes	Yes	Yes	No
Teratoma	Yes	Yes	No	Yes	No
Denervated limb	No	Yes	Paralysed	Yes	Unusual
Explant	No	No	No	No	Always

It will be noted that the only factor which is constantly associated with persistence of union between the two elements is absence of calcification in the cartilage. On this evidence it would seem that the completion of joint formation occurs at a time when the cartilages have reached a certain stage of development, and that a further accompaniment of this stage is the occurrence of calcification. The failure of both calcification and joint formation in the tissue explants occurs because they are not, at this stage, in a normal environment.

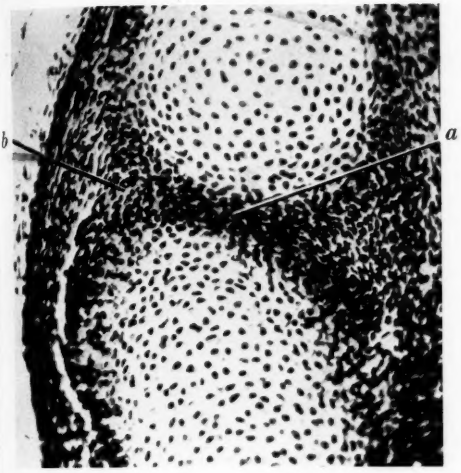
Carey, in 1922, stated that the joint was completed by liquefaction of the tissue (termed by him "mucoid") uniting the two elements. This observation has been confirmed in the present investigation by the examination of the joints of the upper limb, particularly the elbow, in rats during the few days following birth. At this period, when the joint surfaces are separating, the matrix of the primitive cartilage uniting the two bone elements loses its staining reaction. The process is most marked near the edges where the matrix is exposed to the synovial fluid present in the circumferential cavity. Separation occurs by progressive liquefaction of the degenerate matrix from the margins inwards. Fig. 9 represents the appearances seen in the central part of the bond of union between the ulna and the humerus in a newly born rat. It can be seen that the young cartilage is here normal and gives the typical staining reactions. Fig. 10 represents a section through the marginal part of the same joint nearer the synovial cavity. Here the matrix is degenerated and stains with great difficulty. Fig. 11 shows a section through the wrist joint where the liquefaction of the matrix and ingress of synovial fluid have occurred almost to the centre of the joint. These processes take place in the white rat during the few days immediately after birth, and in the human embryo about the fifth month. The cartilage cells exposed in the process of liquefaction become flattened where a considerable part of their surface is in relation to the synovial fluid, but where they are still protected by matrix over the greater part of their surface they remain spherical for some time. The reason for the liquefaction of the matrix along the joint line at this period is unknown. It occurs on the attainment of a certain stage of development of the cartilage which is, in some situations, associated with the appearance of calcification. It is possible that attempts at movement in the joint at this critical period may be of assistance in the separation of the two bones.

This investigation has directed attention to further problems on which work is now being carried out. The first is the explanation of the fact that the costal elements of the vertebrae in the thoracic region form joints with the other elements, whereas in the other regions they become incorporated in the vertebrae. Secondly it seems likely that the behaviour of undifferentiated mesenchyme under various stresses and strains in controlled experimental conditions such as tissue culture offers a method for elucidating further the factors which determine the appearance of the cavity in synovial joints.

In conclusion I must record my thanks to my technical assistant Mr G. A. Walker who has been responsible for the preparation of the serial sections which have been necessary in this investigation and who has prepared for me the photographic illustrations.

SUMMARY

1. The initial stages in the formation of synovial joints show that counter-pressure of the two cartilage elements on the intervening mesenchymal joint disk may be responsible for the progressive chondrification of the disk and



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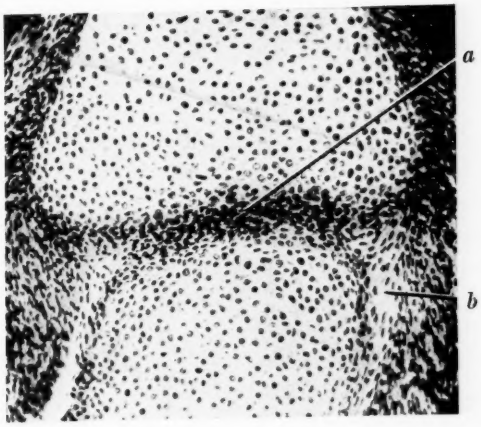

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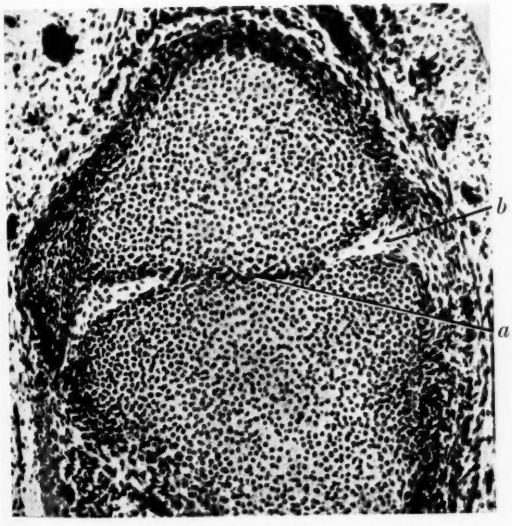

Fig. 3.

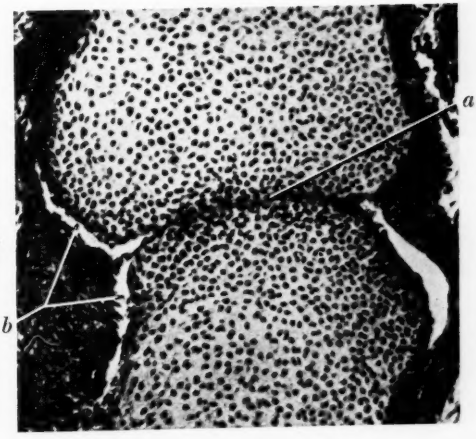

Fig. 4.

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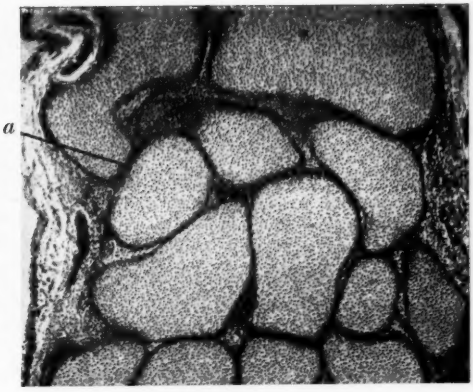
Fig. 5.



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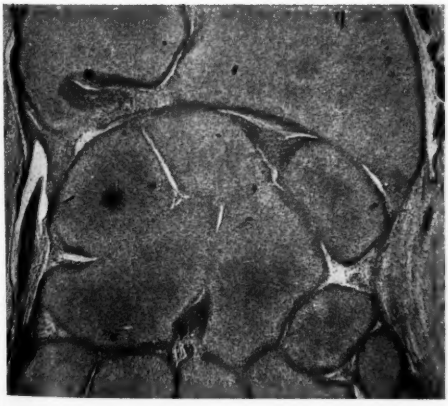

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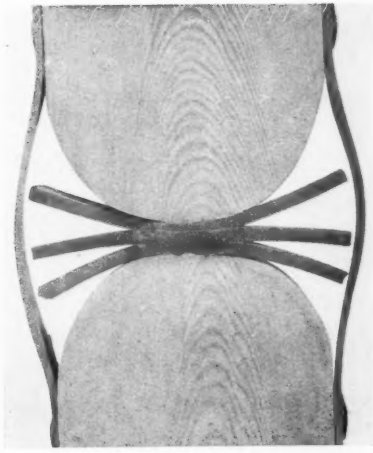

Fig. 8.

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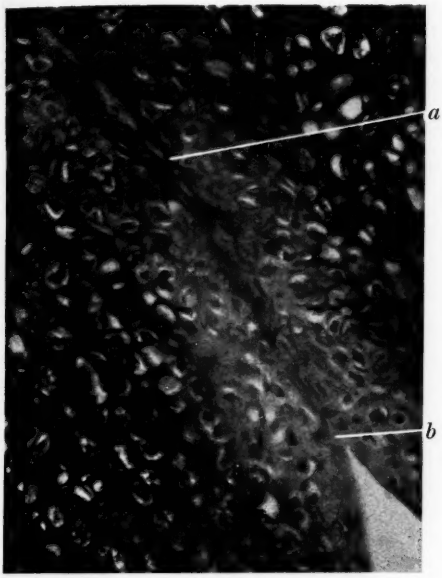

Fig. 9.

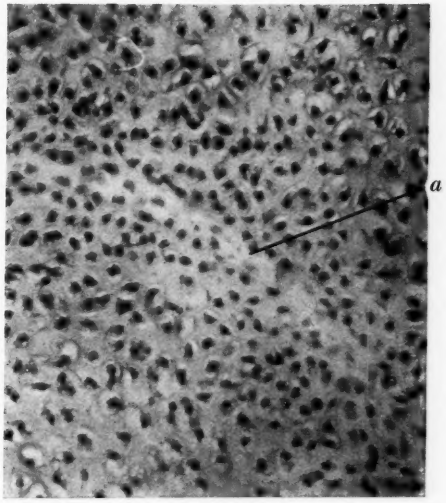

Fig. 10.

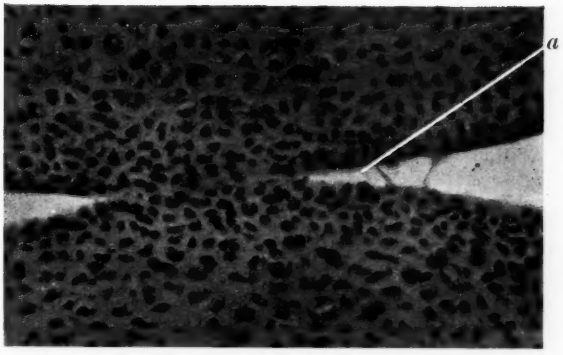

Fig. 11.

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for the appearance of the circumferentially placed synovial cavity. Where pressure on the disk is removed it does not chondrify and this accounts for the presence of intra-articular disks at the wrist and at the two ends of the clavicle.

2. After chondrification of the disk, the two elements in the joint are united for a time by primitive cartilage.

3. The solution of continuity in the later stages of joint formation is accomplished by liquefaction of the matrix of the primitive cartilage uniting the two bones. The cause of the liquefaction is not explained.

Movement alone does not cause breakdown of the bond of union between the two cartilage elements, but it may play an ancillary part in the process.

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EXPLANATION OF PLATES I–III

Plate I

Fig. 1. Coronal section of an interphalangeal joint at the 30 mm. stage. ×200. *a*, joint disk; *b*, looser mesenchyme in circumferential part.

Fig. 2. Interphalangeal joint at 45 mm. stage. ×180. *a*, joint disk; *b*, synovial cavity forming in circumferential part of disk.

Fig. 3. Interphalangeal joint at 63 mm. stage. ×150. *a*, joint disk reduced to two cells in thickness; *b*, synovial cavity.

Fig. 4. Interphalangeal joint at 72 mm. stage. ×185. *a*, disk reduced to single layer of cells; *b*, synovial cavity.

Plate II

Fig. 5. Interphalangeal joint at 125 mm. stage. ×60. *a*, Liquefaction of matrix in marginal part of bond of union.

Fig. 6. Wrist region, 30 mm. stage. ×55. *a*, joint disk between ulnar styloid and triquetral.

Fig. 7. Wrist region, 125 mm. stage. ×20.

Fig. 8. Model illustrating effect of pressure on a laminated disk formed of sheets of rubber.

Plate III

Fig. 9. Section of elbow of newly born rat. (Thionin.) ×340. *a*, continuity of matrix across joint line; *b*, liquefaction of matrix at edge near synovial cavity.

Fig. 10. Section of elbow of newly born rat, nearer the edge of the bond of union than Fig. 9. ×340. *a*, degenerating matrix.

Fig. 11. The process of separation almost completed. ×340. *a*, liquefaction of the matrix.

THE EVOLUTION OF THE HAVERSIAN PATTERN IN BONE

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INTRODUCTION

Most of the work done on the bone of vertebrates has been confined to the study of osteogenesis, or to a definition of the structure of the matrix and the arrangement of the vascular canal systems. The significance of the shape and arrangement of the lacunae and canaliculi appears to have been overlooked, except by a few authors who in the course of other work have commented upon the appearance of the osteocytes. Prominent among these is Stephan, who, however, confined his work to fishes.

An examination of the bone of fishes showed that the arrangement of the lacunae and canaliculi, reflecting as it must the disposition of the contained cells, differed considerably from the cell-pattern of the mammalian Haversian system. It seemed possible that the arrangement in the fishes represented the more primitive state, and that the difference indicated a trend in the evolution of the osteocyte, stages of which might be observed in vertebrates still extant. Additional support is lent to this view by the observations of Fish (1939), who showed that when the undifferentiated mesenchyme cells of mammalian bone were stimulated by toxic, mechanical or neoplastic influences they produced under stress a tissue having a cell pattern similar to that found in the Reptilia. He suggested that this might be a reversion to primitive type.

In order to determine whether intermediate stages could be observed, bone from a number of representative vertebrates was examined. It was found that a gradual modification of cell pattern had taken place throughout the series and that even between the most closely allied species minute variations could often be detected. The latter, however, did not appear to be significant, and it was therefore decided to present in some detail a description of the lacunae and canaliculi of representative vertebrates, rather than to describe a large number of species in general terms.

METHOD

To facilitate interpretation, bone from anatomically analogous parts was examined wherever available; and for the most part sections were made of the diaphyses of the long bones, the epiphyses being avoided. Since ordinary decalcified sections do not show the arrangement of lacunae and canaliculi in any great detail, ground sections were made in each case, and were controlled

by decalcified sections in order to show more clearly the condition of the cells, the structure of the matrix and the presence of Howship's lacunae or cement lines. In fresh ground sections it was found that none of the usual stains would permeate the matrix of mature bone, but that methylene blue and basic fuchsin diffused readily into the lacunae and canaliculi. In this investigation the latter stain was used most generally.

In some cases, when the ground sections were difficult to interpret, they were photographed and drawn with a modification of the camera lucida, and then unmounted, decalcified and stained afresh with haematoxylin and eosin or any other suitable combination.

I. FISHES

Stephan's work (1900) led him to suppose that although the bones of the most primitive fish possessed cells there was a definite degenerative process taking place culminating in the bone of the most specialized species, which as in the case of the majority of the Teleostei is acellular.

In the bone of *Protopterus* or *Lepidosiren* there is a comparatively large number of osteocytes, which occupy large irregularly shaped lacunae scattered sporadically throughout the matrix. The canaliculi are both large and numerous, but, in view of their haphazard course, they appear to form relatively few connections with those of neighbouring lacunae. A similar condition occurs in *Polypterus* where the canaliculi are so numerous that they may completely mask the cell.

It was observed that even in the branchial arches, which are laid down in much the same way as the long bones of the Amphibia (Haines, 1934), the shape and arrangement of the lacunae, and more especially the canaliculi, nevertheless remain extremely irregular.

In the less specialized Teleostei the lacunae, which are present, clearly resemble in shape and arrangement those found in the more primitive forms, but although the canaliculi also branch irregularly, there is a reduction in their number. This modification is carried still further in *Lepidosteus*, *Accipenser* and many of the Physostomes, where the canaliculi of any particular lacuna tend to be confined to one plane.

In the Salmonidae this morphological trend is even more noticeable. Here, although there is still a comparatively large number of lacunae, there is a great reduction in the number of canaliculi, and the lacunae present a regular oval outline rather than the usual stellate contour (Fig. 1). In other cases there are short processes leaving the lacunae (Fig. 2), but since they usually appear to terminate abruptly in the matrix and are very rarely observed to form a connection between neighbouring lacunae, they can do little to facilitate the nutritional processes of the cells. Stephan was in fact inclined to regard the osteocytes which do occur in this tissue as little more than vestigial cells.

Finally in the majority of the Physoclisti the bone is completely acellular.

An examination of serial decalcified sections of the bones of primitive fishes shows that a comparatively large proportion of the lacunae are empty, the

osteocytes presumably having died and undergone autolysis. Stephan (1900) considered that the irregularity of the canaliculi and their failure to anastomose in the bone of fishes deprived the cells of tissue fluid upon the free movement of

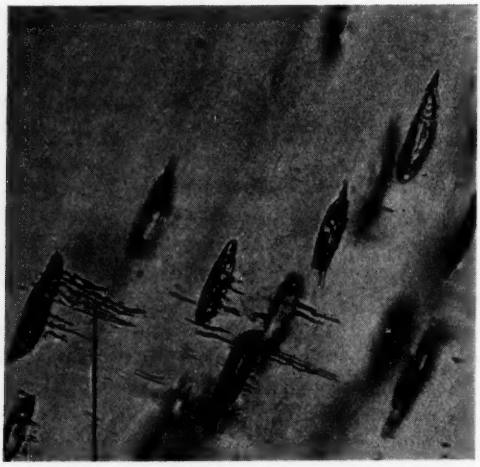

Fig. 1. Longitudinal ground section from vertebra of *Salmo fario*. Although the lacunae possess a certain uniformity there are no canaliculi. ×480.

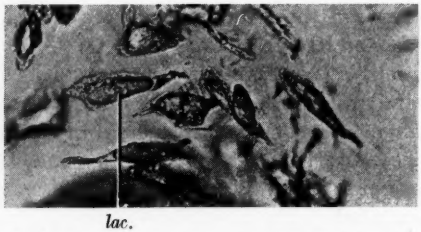

Fig. 2. Longitudinal ground section from vertebra of *Salmo fario*. The lacunae possess short degenerate canaliculi. ×480.

which they rely for nourishment and for the disposal of waste matter. He did not, however, appear to associate this observation with the fact that the more inaccessible cells had actually disappeared from their lacunae.

II. AMPHIBIA

The bone of the Amphibia may be studied in a primitive and in a highly specialized type—*Megalobatrachus* and *Rana temporaria* respectively. The arrangement of the cells in the higher members of the Anura and Urodoles is so similar that it will be unnecessary to describe both.

Megalobatrachus

The long bones of *Megalobatrachus* assume a simple tubular form uncomplicated by the presence of any vascular canal systems and consist of compact periosteal tissue laid down in layers around the marrow cavity (Eggeling, 1911).

The typical arrangement of cells may be seen in Fig. 3. The lacunae, which are comparatively few in number, resemble those of the primitive fishes very closely. Although they are usually elongated in the direction of the axis of the

Fig. 3. Transverse ground section from diaphysis of humerus of *Megalobatrachus*. The canaliculi show a tendency to run radially. ×200.

bone, their asymmetry is not very pronounced, and whether examined in longitudinal or transverse section, they retain their characteristic irregular stellate appearance. They occur haphazardly throughout the matrix and show no signs of being arranged in layers as the bone is laid down.

The canaliculi issue from all parts of the surface of the lacunae but differ from those of *Polypterus* in that they exhibit a preferential orientation and tend to run radially from the marrow cavity to the periosteum. That their arrangement is, however, by no means stereotyped may be seen from Fig. 3, and if their course be traced in thick sections it will be found that few connections are formed with neighbouring lacunae.

Decalcified sections show that although healthy osteocytes appear to occupy the majority of the lacunae, cases occur comparatively frequently where the cells appear to have died and the lacunae are empty.

Rana temporaria

In *Rana*, the bone represents the perfection of that type of tissue which is seen in the primitive state in *Megalobatrachus*.

The lacunae are regular elongated plate-like cavities arranged so that their long axes run in a direction parallel to that of the marrow cavity. Their distribution throughout the matrix is more regular than any hitherto described, and

Fig. 4. Longitudinal ground section from diaphysis of tibio-fibula of *Rana temporaria*. The regular arrangement of lacunae and canaliculi is shown. ×390.

the cells tend to lie in rows along the lines separating the laminae. The canaliculi, which present a perfectly regular appearance (Fig. 4), run radially from marrow cavity to periosteum, and since they anastomose to a greater extent with those of neighbouring lacunae they are correspondingly shorter in length in comparison with those of *Megalobatrachus*.

The lacunae, moreover, by virtue of their longitudinal orientation, present a larger surface area towards the marrow cavity and thus enable more canaliculi to communicate with each cell than would be possible in the more primitive types.

Although this tissue, therefore, is for the most part very well organized, it does occasionally include lacunae such as those seen in Fig. 5. They occur constantly in ground sections amongst perfectly normal cells, and are remarkable in that they do not take up the stain; nor are they observed to contain osteocytes when the section is decalcified and restained. The canaliculi are more tortuous and irregular than usual and do not often appear to communicate with those of neighbouring lacunae.

Despite these more primitive and perhaps vestigial lacunae, the diaphysis of the long bones of the higher Amphibia, as exemplified by *Rana*, is comparable in its arrangement to a single Haversian system, though of course on a larger scale. The cells in it are in fact irrigated only less effectively in as much as those at the centre are farther removed from their source of vascular supply, and must therefore receive tissue fluid which has already been depleted and contaminated by a larger number of cells.

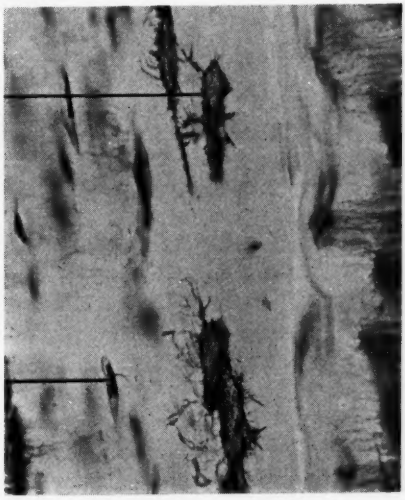

Fig. 5. Longitudinal ground section from diaphysis of tibio-fibula of *Rana temporaria*. Lacunae containing normal osteocytes are shown adjoining those which do not contain osteocytes (*e.lac.*). The latter are refractile and their canaliculi are very irregularly arranged. ×400.

III. REPTILES

Bony tissue in four representative types of reptiles may be described. These include the primitive species *Sphenodon*, a tortoise (*Testudo graeca*), a lizard (*Iguana tuberculata*) and a crocodile (*Crocodilus niloticus*).

Sphenodon

The long bones of *Sphenodon* possess no definite vascular canals and assume the simple tubular form characteristic of the Amphibia.

The arrangement of the cells is remarkably similar to that of *Megalobatrachus*, and although the cells show a slightly greater tendency towards elongation they possess the same stellate and very irregular shape (Fig. 6). The canaliculi, which apparently issue at all angles from the lacunae, tend on the whole to run radially from the marrow cavity to the periosteum, although considerable

individual variation is to be found. Again by tracing the course of canaliculi in thick sections it is possible to determine that many fewer connections between neighbouring lacunae are formed than appear in the bone of *Rana*.

Iguana tuberculata

The long bones of the adult lizard are similar to those of the frog in that the superficial layers of the diaphysis consist of periosteal bone which contains no vascular canals, but here the resemblance ends, since absorption has taken place around the marrow cavity with the resultant formation of large excavated canals within which secondary bone is deposited (Demeter & Matyas, 1928).

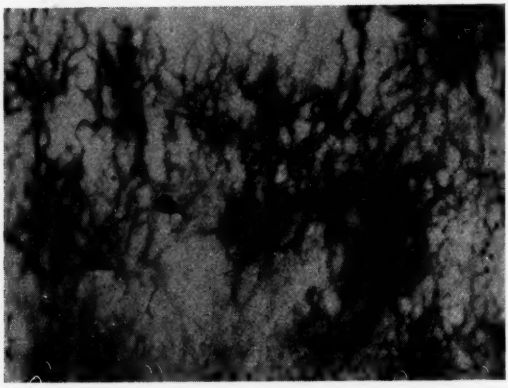

Fig. 6. Transverse ground section from diaphysis of ulna of *Sphenodon*. The irregular arrangement of lacunae and canaliculi is shown. ×420.

The arrangement of the cells in the periosteal bone is similar to that found in the frog. Thus the lacunae are regular plate-like cavities, while the canaliculi run radially from the periosteum towards the marrow cavity, forming frequent connections between neighbouring lacunae (Fig. 7). In contrast with the highly organized appearance of the periosteal bone, however, the arrangement of the cells in the secondarily deposited tissue is as primitive as any which may be found in fishes such as *Protopterus* or *Lepidosiren*.

The lacunae are very unevenly distributed, occasional patches of matrix being almost acellular, whereas in adjoining areas the lacunae are almost confluent. Individual lacunae vary considerably in size and shape, and whether seen in longitudinal or transverse section retain their characteristic very irregular appearance (Fig. 8). The canaliculi appear to follow a very haphazard course, and in many cases the cell seems to be completely isolated in the matrix. Decalcified sections show that although there is a comparatively large number of living osteocytes in the tissue the proportion of empty lacunae is

Fig. 7. Transverse ground section from diaphysis of fibula of *Iguana tuberculata*. The outer layer of regular periosteal bone (*per.*) is separated by a cement line from the endosteal bone (*end.*) which surrounds the excavated canals (*e.cnl.*). × 126.

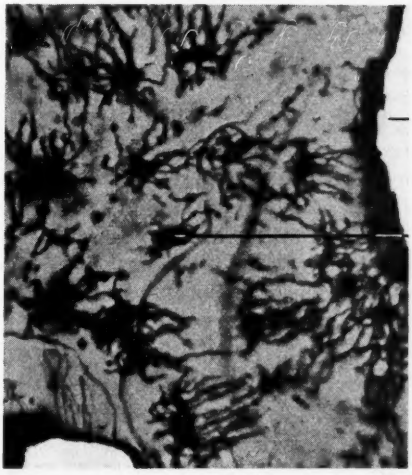

Fig. 8. Transverse ground section from diaphysis of fibula of *Iguana tuberculata*, showing the irregular arrangement of lacunae and canaliculi in endosteal bone. × 420.

unusually high, and noticeably greater than in the periosteal bone. It is significant that when the osteocytes are situated near a canal they usually present a perfectly healthy appearance, but when either cement lines, or a large area of matrix intervenes between a lacuna and the nearest blood vessel, it will more often than not appear to be empty (Fig. 9).

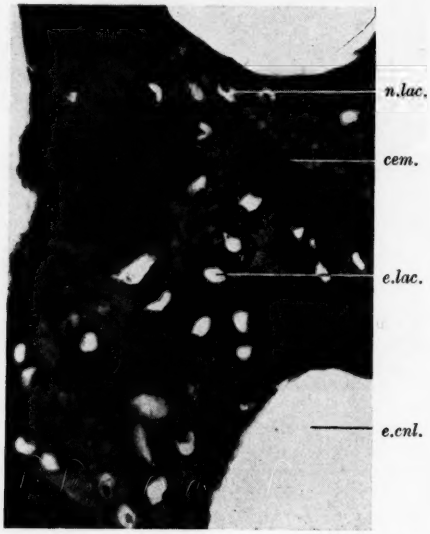

Fig. 9. Transverse decalcified section from diaphysis of tibia of *Iguana tuberculata*, showing that the lacunae, which are enclosed within the cement lines, are with one exception empty, whereas those close to and not separated from the excavated canals contain osteocytes. Stained Van Gieson. × 348.

The contrast between this type of tissue, which must be regarded as being a very primitive manifestation of the Haversian system, and that of the periosteal bone is very marked. A fuller investigation, however, would be necessary before deciding whether the endosteal bone is merely degenerate, indicating an atavistic trend, or whether it is a survival of primitive origin, marking a stage in the development of the Haversian system.

Crocodilus niloticus

An intermediate stage is seen in the crocodile whose long bones differ from those of *Iguana* in that the outer layer of periosteal tissue contains a large number of nutrient canals. It is important to distinguish between these canals, which originate in the periosteum, and are termed "Infallungscanäle" by Weidenreich (1923), and excavated canals, which are of endosteal origin. Both

are observed in the crocodile, for in the adult resorption takes place, as in *Iguana*, and fibrous bone is deposited in the new canals thus formed (Fig. 10).

Both periosteal and endosteal tissue is remarkable for the lack of organization of the cellular elements. In the periosteal bone the lacunae resemble those of *Megalobatrachus* rather than *Iguana* both in their shape, which in spite of a tendency towards elongation remains far from regular, and in their very haphazard occurrence throughout the matrix, where they are not orientated in relation to the nutrient canals (Fig. 11). The canaliculi, although tending to run radially, retain much of the irregularity characteristic of the more primitive forms. When the cells lie in the immediate neighbourhood of the canals, the canaliculi usually form a means of communication with them, but this bears no resemblance to the Haversian system, and it seems probable that only the osteocytes which are very close to such a nutrient supply will receive the full benefit from it.

The cells of the endosteal bone of the crocodile (Fig. 12) are similar to those of *Iguana*. There is the same lack of organization, and the canaliculi form relatively few connections between the osteocytes and the absorption canals. The distribution of the lacunae throughout the matrix is very irregular though less so than in *Iguana*, and a decalcified section indicates that a high proportion of the lacunae contain no living cells.

Presumably this bone shows an advance on that of *Iguana* in that the periosteal tissue contains quite a rich blood-vascular supply, but the cells can gain little advantage from this modification, owing to the irregular arrangement of their nutrient canaliculi.

Testudo graeca

The long bones of the tortoise are constructed in the same way as those of the crocodile (Demeter & Matyas, 1928). In the adult the diaphysis consists of an outer layer of periosteal bone, while towards the marrow cavity excavated canals have formed and endosteal bone is deposited (Fig. 13).

The arrangement of cells in the periosteal bone of the tortoise and crocodile is very similar. The same lack of organization is still apparent in the irregular shape and distribution of the lacunae, which also appear to bear little relationship to the nutrient canals. The canaliculi although tending to run radially show considerable individual deviation, and are scarcely more effective than those of the crocodile in connecting distant lacunae with the blood vascular supply.

The endosteal tissue differs from any described above in that it is laid down in the form of Haversian systems (Figs. 13, 14). The lacunae, which are elongated, plate-like cavities, are arranged concentrically around the canals, and tend to lie between the bone lamellae.

The canaliculi, which run radially, present a perfectly regular appearance, and form a means of communication between the canal and the most distant

Fig. 10. Transverse ground section from diaphysis of ulna of *Crocodilus niloticus*. Towards the periosteum there is periosteal bone containing nutrient canals (*nut.*) whilst around the marrow cavity secondary endosteal bone is deposited within the excavated canals. ×55.

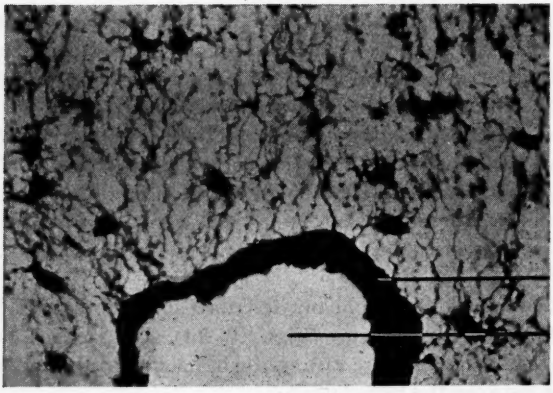

Fig. 11. Transverse ground section from diaphysis of ulna of *Crocodilus niloticus*, showing the irregular arrangement of lacunae and canaliculi in periosteal bone. ×400.

Fig. 12. Transverse ground section from diaphysis of ulna of *Crocodilus niloticus*, showing endosteal bone. ×440.

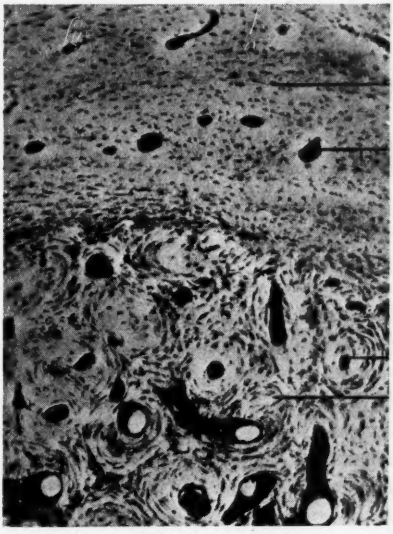

Fig. 13. Transverse ground section from diaphysis of humerus of *Testudo graeca*. The endosteal bone is laid down as Haversian systems. ×56.

cells. In comparison with the bone of the mammals an individual system tends to contain fewer osteocytes, and irregular patches of tissue of endosteal or more rarely periosteal origin are of rather more frequent occurrence. The bone of the tortoise thus occupies a position between that of the crocodile and the mammal, for although Haversian systems are laid down in the endosteal bone, the cells of the periosteal tissue retain their more primitive arrangement.

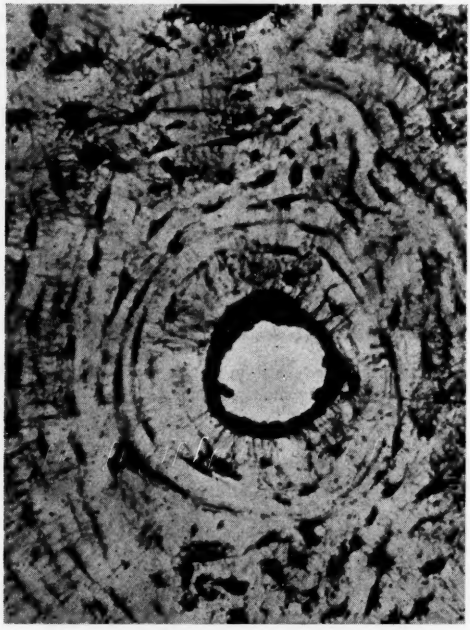

Fig. 14. Transverse ground section from diaphysis of humerus of *Testudo graeca*, to show Haversian system of endosteal bone with adjoining irregular lacunae. × 275.

IV. MAMMALS

During the development of the mammalian long bones there are stages in which the arrangement of cells is directly comparable with that found in the adults of more primitive species. Thus when the diaphysis consists of periosteal bone containing nutrient canals, and before extensive absorption and redisposition of secondary bone has commenced, the organization of the lacunae and canaliculi is very similar to that found in the crocodile. The distribution and shape of the cells in both periosteal and endosteal tissue is very irregular and in both cases they bear little relationship to the canals. When absorption and redisposition become further advanced and Haversian systems have re-

placed much of the endosteal bone, the bone passes through a phase when it closely resembles the bone of *Testudo*.

In the adult mammal, however, the lacunae and canaliculi achieve a degree of regularity and uniformity of structure and arrangement unequalled in the lower vertebrates. In many species the embryonic bone is almost entirely replaced by endosteal Haversian systems, the remnant of periosteal bone appearing only as a shallow layer at the periphery of the diaphysis. In other types such as *Equus*, periosteal bone with nutrient canals is persistent, but nevertheless differs from that described in the reptiles inasmuch as secondary bone is deposited around the canals in such a way that the arrangement of the cells is indistinguishable from that observed in the endosteal Haversian system.

The only bony tissue in the mammal which contains cells where this organization does not prevail is found in the alveolus surrounding the teeth. This bone is of a transitory nature in that it is absorbed when the teeth are lost. The lacunae which occur here are very irregular in shape and distribution and appear to be very imperfectly connected by means of canaliculi. Also the cementum of the teeth resembles primitive bone and it is significant that in the interior of the cementum the osteocytes are liable to die and leave empty lacunae (Ross, 1935).

SUMMARY AND CONCLUSIONS

This investigation has shown that, from the chaotic arrangement of the cells and their processes as observed in primitive bone, order and continuity gradually emerge and reach perfection in the mammals.

Throughout the higher vertebrates there is a definite trend towards the establishment of a regular cell pattern. The development of bone as an organized tissue does not however follow exactly the same course in the various classes, and it has been observed that in the fishes evolution has taken the opposite direction, since the bone of the more specialized species tends to become acellular.

In the bone of the higher members of the Anura and Urodeles, however, the haphazard arrangement of cells observed in the bone of most of the fishes and in the primitive Amphibia has already resolved itself into a pattern directly comparable with that found in a single Haversian system of bone. The appearance of definite canal systems is first noted in the more specialized Reptilia, and although the cellular arrangement of the secondarily deposited tissue is very primitive in the lizard, in other reptiles such as the tortoise the bone is deposited round the vessels in the canals in the true Haversian pattern.

The evolutionary nature of these modifications is emphasized by the way in which the embryonic bone of the mammals reproduces to a certain extent an arrangement of cells comparable with that observed in these lower orders. Even in the adult mammal, calcified tissue of repair which is produced in

response to abnormal stimuli affecting undifferentiated mesenchyme cells bears a marked resemblance to the primitive bone of the Amphibia and the lizard.

The value of such a developmental process is necessarily obscured by the gap which exists in our knowledge of the physiology of the bone matrix. The matrix of adult bone, although impermeable to ordinary dyes, does apparently require the presence of moisture if only to provide for the hydration of its organic content, and this involves the provision of numerous minute irrigation canals. The presence of cells at frequent intervals is, therefore, also a contingent necessity in order to circulate the tissue fluid in these irrigation canaliculi, for these cells, by their metabolism, must set up diffusion currents which achieve this purpose. Owing to the relative impermeability of the matrix, however, they are in turn entirely dependent on the canals as their sole means of obtaining tissue fluids for nourishment and for the elimination of their waste products. It would therefore seem probable that it is the lack of organization of the canaliculi in primitive bone, and the consequent "isolation" of many lacunae, that results in the death of a high proportion of osteocytes. In the bone of the higher vertebrates every lacuna is eventually connected with a Haversian canal by means of regularly arranged canaliculi, and as a result cells seldom die and empty lacunae are very rarely observed.

Whatever purpose this elaboration in the pattern of the matrix may ultimately be shown to serve, it is evident that the perfect geometric design of the canaliculi in the Haversian system of adult mammalian bone does provide for more uniform and complete irrigation of the matrix; and that the empty lacunae of primitive bone are an indication of incomplete adaptation of osteocytic elements to their environment. Further evidence of the superior efficiency of Haversian bone may perhaps be found in the fact that the callus of a fracture which resembles primitive bone is rapidly replaced by true Haversian bone when the limb regains function, but is not so readily replaced in an amputation stump which has lost its function (Fish, 1939).

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KEY TO LETTERING

cem.	cement line	*lac.*	lacunae
e.lac.	empty lacunae	*n.lac.*	normal lacunae containing osteocytes
end.	endosteal bone (secondarily deposited)	*nut.*	nutrient canals
e.cnl.	excavated canals	*per.*	periosteal bone
h.c.	Haversian canal	*z.i.c.*	zone of incomplete calcification where stain has penetrated the matrix
s.f.	Sharpey's fibres		

N.B. Unless otherwise stated the sections were stained with basic fuchsin.

AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF THE AMPHIBIAN CLOACA

By R. J. O'CONNOR

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INTRODUCTION

Spemann (1938) and others have shown that in the development of the amphibian optic lens there is a combination of the self-differentiating power of the lens ectoderm with the lens-inducing power of the optic cup. Further, in different amphibian species it has been shown that there is a considerable variation in the relative importance of these two developmental factors. Recent work on the development of the mesonephros (Waddington, 1937; O'Connor, 1939) has shown that in the development of this structure there is a similar co-operation of developmental forces. The development of the mesonephros is dependent partly on the self-differentiating power of the mesonephric cells and partly on a stimulating influence due to the contact of these cells with the pronephric duct. Here, as in the case of the optic lens, there is a variation in different species of the relative importance of the self-differentiating power and the stimulating effect of the adjacent structure.

The two structures concerned in the development of the optic lens on the one hand and the mesonephros on the other are in a close functional and topographical relationship, and the possibility is suggested that when any two structures are in such a relationship they may have a similar interaction in development. Such a relationship exists between the pronephric duct and the cloaca in Amphibia. In *Urodeles* the pronephric duct is formed by a caudal growth of the pronephric rudiment which appears in the third to seventh segments. The pronephric duct meets the cloaca at the dorsal aspect of its cranial wall and there forms a patent union. At the point of union there is a thinning of the cloacal wall, giving rise to a diverticulum of the cloacal cavity on either side. Subsequently these diverticula become more pronounced, involving both the cloacal wall and the cloacal cavity, and receive the pronephric ducts at their apices. The formation of these diverticula would thus appear to be closely related to the union of the pronephric ducts with the cloaca and experiments were performed to investigate the development of the cloaca in the absence of one or both pronephric ducts and particularly to investigate the development of the cloacal diverticula in such circumstances.

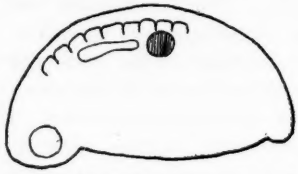
MATERIAL AND TECHNIQUE

The material used consisted of the embryos of *Triton taeniatus*, *Pleurodeles waltlii* and a species of *Amblystoma* the specific name of which is not certain.

Experiments performed were of two main types:

(*a*) Localized excisions and transplantations of tissues. In this case the technique used has been described in a previous paper (O'Connor, 1939). In these experiments the commonest procedure was to prevent the caudal growth of the pronephric rudiment that gives rise to the pronephric duct. A transplant was placed at the caudal limit of the pronephric rudiment soon after it appeared and when it was confined to the third to seventh segments. The situation of the transplant is indicated in Text-fig. 1. It consisted of any convenient tissue taken from another embryo.

(*b*) Vital staining experiments. Nile blue sulphate was applied to localized regions of the embryo by means of small portions of agar soaked in the dye. The method of application is described by Stone (1931).



Text-fig. 1. *Amblystoma* at the stage of the first appearance of the pronephric rudiment. The shaded area indicates the site of the transplant to prevent the formation of the pronephric duct.

DEVELOPMENT OF THE CLOACA IN THE ABSENCE OF ONE PRONEPHRIC DUCT

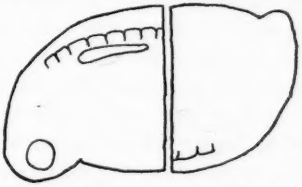
The successful placing of a transplant as described above resulted in the non-formation of the pronephric duct on the operated side and consequently the development of the cloaca could be followed in the absence of one pronephric duct. In all such experiments the cloaca developed as a symmetrical structure. Thinning of the cloacal wall took place at the same site and at the same time as on the normal side, and the formation of the diverticula of the cloacal wall was equal on the two sides (Pl. I, fig. 1). A similar result was obtained in all of the three species examined. Therefore, in the absence of the pronephric duct on one side, the cloaca undergoes a normal development, and in all three species examined the formation of the cloacal diverticula is independent of the pronephric duct on the same side.

DEVELOPMENT OF THE CLOACA IN THE ABSENCE OF BOTH PRONEPHRIC DUCTS

The possibility exists, however, that in the absence of one pronephric duct the symmetrical development of cloacal diverticula may be due to some influence exerted on both sides of the cloaca by the remaining pronephric

duct. Although this would seem somewhat unlikely its exclusion is possible by examining the development of the cloaca in the absence of both pronephric ducts. Experiments for this purpose were most conveniently performed by taking embryos at the stage of the first appearance of the pronephric rudiment and dividing them by a dorso-ventral cut in such a way that the caudal portion contained none of the pronephric rudiment. The two portions were then reunited with the caudal half reversed as is indicated in Text-fig. 2. Healing was usually successful, and the abnormal arrangement of the structures in the embryo prevented the extension of the pronephric ducts into the caudal portion of the embryo so that the cloaca underwent subsequent development in the absence of both pronephric ducts.

In all cases and in all species examined the cloaca underwent a normal development and cloacal diverticula were formed as in normal embryos (Pl. I, fig. 2). It is certain therefore that, in the three species of Amphibia examined, the development of the cloaca is independent of the pronephric ducts and the cloacal diverticula to which the ducts join are formed independently of them. These observations suggest that in all amphibian species



Text-fig. 2. Division and reunion of an embryo to prevent the formation of both pronephric ducts in the caudal portion.

these cloacal diverticula are formed independently of the pronephric ducts. However, such a generalization should be conditional on similar findings being recorded for other species, especially as the work of Boyden (1924) shows that differentiation of the middle chamber of the avian cloaca depends on the contact of the pronephric or Wolffian ducts.

ROLE OF THE CLOACAL DIVERTICULA IN THE UNION OF THE PRONEPHRIC DUCT AND THE CLOACA

The modifications of the cloacal wall that lead to the formation of diverticula begin at the time the pronephric duct meets the cloaca, and it would thus appear that the formation of diverticula is essential to the patent union of the pronephric duct and the cloaca. Experiments were therefore performed to investigate in more detail the manner of union of the cloaca and pronephric duct and to investigate the role of the diverticula in the communication of the two structures. These investigations have been planned to answer the following two questions:

(*a*) Can the pronephric duct form a patent union with other structures than the cloaca?

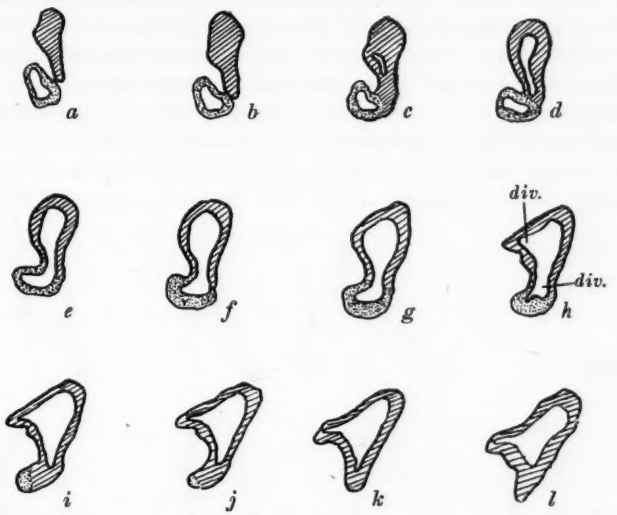
(*b*) Can the pronephric duct form a patent union with any other portion of the cloacal wall than the site of formation of the cloacal diverticula?

(*a*) ***Union of the pronephric duct with structures other than the cloaca.*** Many experiments were performed of the type indicated in Text-fig. 1 in order to prevent the caudal growth of the pronephric duct. These resulted in certain modifications of the pronephric duct immediately cranial to the transplant. In some cases these modifications consisted of a patent union of the pronephric duct either with the exterior or with the gut. These abnormal unions have been described and figured in a previous paper (O'Connor, 1939). However, the occurrence of such communications was unusual and they were only found in 4 % of 150 cases examined; thus they do not indicate more than an extremely limited capacity of the pronephric duct to unite with abnormal structures. These results are supported by an experiment in which an embryo of *Triton taeniatus* was divided as shown in Text-fig. 2. The caudal portion was removed and the raw surface of the cranial half covered with a flap of ectoderm. As the pronephric ducts grew caudally they came up against the ectoderm and could grow no further caudally. The embryo was fixed 7 days after the operation and sectioned longitudinally. A section passing along the left pronephric duct is shown in Pl. I, fig. 3, where it is seen that the pronephric duct comes up against the ectoderm but has made no communication with it. This observation was confirmed by examination of all the sections, when it was seen that the same findings were present in the case of the duct of the opposite side. These results, then, confirm the conclusion that the pronephric duct has not any great power to unite with structures other than the cloaca.

(*b*) ***Union of the pronephric duct with the cloacal wall.*** In this investigation experiments were performed where the cloaca and surrounding tissues from one embryo were transplanted into another in the situation and at the stage indicated in Text-fig. 1. As the pronephric duct of the host embryo grew caudally on the operated side it came into contact with the transplant and its further growth was prevented. In some cases the surrounding tissues transplanted with the cloaca prevented contact between the duct and the cloacal wall, but in twenty-five cases such a contact between the two structures resulted, these consisting of twelve cases in *Amblystoma* and thirteen in *Triton taeniatus*. In ten cases there was a patent union between the two structures, while in the remaining fifteen there was merely a contact between the two structures and the pronephric duct did not open into the transplanted cloaca. These cases were examined in detail, and since the findings were similar they can be illustrated by one case from each group. Where a patent union formed it was found that the transplanted cloaca formed diverticula and that the pronephric duct opened into one of these diverticula. This is illustrated by a series of camera lucida drawings of sections showing the entrance of the pronephric duct into a transplanted cloaca (Text-fig. 3). On the other hand where there was contact between the two structures without a patent union, a series of camera lucida drawings shows that the pronephric duct came into contact

with a portion of the cloacal wall where a diverticulum had not formed (Text-fig. 4).

These results show that, besides only having a very limited ability to unite with structures other than the cloaca, the pronephric duct only unites with the cloaca at a specific locality in the cloacal wall where certain modifications take place leading to the formation of cloacal diverticula.

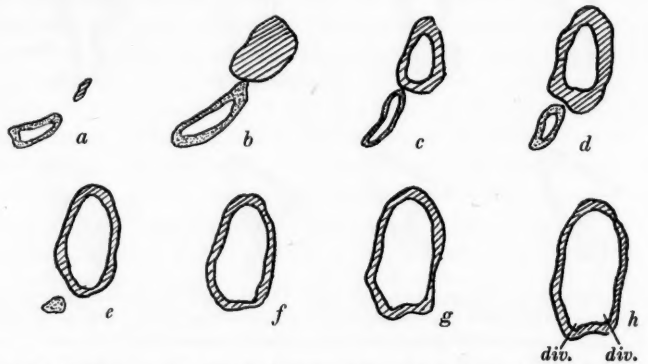


Text-fig. 3. *Amblystoma*, camera lucida drawings. The cloaca from another embryo was transplanted into the left flank 15 days previously. The drawings represent the relationship of the transplanted cloaca and left pronephric duct as seen in consecutive transverse sections cut at 10 μ. Drawing (*a*) represents the most cranial section. The pronephric duct (stippled) approaches the transplanted cloaca (shaded) and opens into it *via* a cloacal diverticulum (*div.*).

THE GROWTH OF THE PRONEPHRIC DUCT SUBSEQUENT TO ITS UNION WITH THE CLOACA

Many experiments in this and other papers have been recorded where the caudal growth of the pronephric rudiment has been prevented. In experiments allowed to develop up to 15 days after the operation the result has been constant—there was no formation of the pronephric duct caudal to the transplant. However, when development was followed for longer periods, it was found that, although the pronephric duct ceased at the transplant and was not seen for a considerable distance caudal to it, there was a structure attached to the cloaca on the operated side, which in its attachment to the cloaca, its position, and histological structure, is identical with the pronephric duct on the non-operated side (see O'Connor, 1939, Text-fig. 2 and Pl. I, fig. 4). The presence of this structure was confined to *Amblystoma* and was not found in a similar series of experiments on *Pleurodeles waltlii* and *Triton taeniatus*. In

Amblystoma this structure was not found before the eighteenth day after operation (representing 22 days' total development) but it became increasingly more frequent in embryos reared for longer periods and was found in all of fifteen cases reared for more than 25 days after operation. The maximal length found in this structure was about 650 μ, and this, as in all cases, represented less than 20 % of the total length of the pronephric duct on the non-operated side. This tag of tissue could not have been derived from the pronephric rudiment, and its position, as well as its time of appearance, makes it extremely unlikely that it is derived from the mesodermal structures at the hinder end of the embryo. It does not appear until the eighteenth day or later, when the mesodermal tissues in the vicinity have already differentiated into muscular and fibrous tissue. The transformation of these into a duct-like structure is scarcely possible, and it is therefore concluded that the cloaca is the most likely source of this structure.

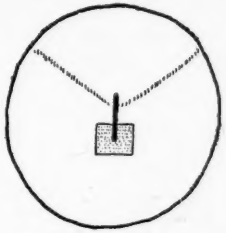


Text-fig. 4. *Amblystoma*, camera lucida drawings of same type of experiment as in Text-fig. 3. The pronephric duct (stippled) approaches the transplanted cloaca (shaded) and comes into contact with a region of the wall where no diverticulum has formed. There is contact between the two structures but not a patent union. (*div.* cloacal diverticulum.)

Attempts were made to test this opinion by the use of vital staining methods. The object of these experiments was to stain the cloaca with Nile blue sulphate prior to the union of the pronephric ducts and to see if subsequently any of the dye placed in the cloaca extended into the hinder ends of the pronephric ducts. Embryos of *Amblystoma* were stained in the earliest neurula stage and the stain applied to the ventral half of the blastopore and the adjacent area as indicated in Text-fig. 5. In the tail-bud stage and later, the dye could be seen in the cloaca, in the region of the tail, and in the myotomes. In sections the dye appeared as blue granules and its precise distribution could be determined. Following this staining procedure a series of embryos was examined soon after the time of union of the pronephric duct with the cloaca. Dye granules could be found throughout the wall of the cloaca as well as in the

caudal somites and adjacent mesoderm. None however could be found in the pronephric ducts. Other embryos were allowed to develop for longer periods, but this led to difficulties in examination as the staining decreased considerably in intensity. Its accurate distribution was thus difficult to determine and negative observations could not be relied upon. However, two cases were found where after twelve days' total development dye was found in the hinder end of the pronephric ducts extending cranially for about 70 μ. It may be mentioned that Bijtel (1931) performed similar experiments and mentions one where dye was found in the pronephric ducts for a distance of about five somites from the cloaca, this being seen after about 12 days' total development.

Although these vital staining experiments cannot be regarded as conclusive, they are in accordance with the findings in experiments where the caudal growth of the pronephric rudiment is prevented. At the time of meeting of the pronephric duct and cloaca there is no formation of a duct-like tag of



Text-fig. 5. *Amblystoma*, early neurula stage. Stippled area indicates the region where the Nile blue sulphate was placed in staining experiments.

tissue, nor is there any extension of the dye into the pronephric duct from the stained cloaca. At later stages of development there is a correspondence between the formation of a tag of duct-like tissue and the presence of dye extending from the cloaca into the hinder end of the pronephric ducts. Therefore in *Amblystoma* it appears justifiable to conclude that, although the pronephric duct is entirely formed by the caudal growth of the pronephric rudiment, there is some evidence to warrant the opinion that the cloaca may subsequently add to its caudal portion.

SUMMARY

1. At the site of union between the cloaca and pronephric duct in Amphibia the cloaca forms diverticula. In *Pleurodeles waltlii*, *Triton taeniatus* and a species of *Amblystoma*, these diverticula are formed independently of the pronephric duct and can be demonstrated in its absence.

2. The pronephric duct has been shown only to unite with the cloaca at the site of formation of these diverticula.

3. In the species of *Amblystoma* investigated there is evidence to show that, although the pronephric duct is primarily formed by the caudal extension of the pronephric rudiment, it may subsequently receive a contribution to its hinder end from the cloaca.

The spawn of *Amblystoma* was kindly supplied by Miss R. M. Renton of the Zoological Department, University College, London. This paper is based on a portion of a thesis accepted for the degree of M.D. by the University of Adelaide, South Australia.

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EXPLANATION OF PLATE I

Fig. 1. *Amblystoma*, transverse section through the cloaca 15 days after operation to prevent the formation of the left pronephric duct. Symmetrical development of the cloaca and cloacal diverticula. (*r.p.* right pronephric duct; *d*, cloacal diverticulum.) × 120.

Fig. 2. *Triton taeniatus*, transverse section through the cloaca 12 days after operation as indicated in Text-fig. 2. Normal development of the cloaca in the absence of both pronephric ducts. (*d*, cloacal diverticulum.) × 140.

Fig. 3. *Triton taeniatus*, longitudinal section 7 days after operation as described in text. The pronephric duct comes up against the ectoderm but does not unite with it. (*p*, pronephric duct.) × 120.

Fig. 4. *Amblystoma*, transverse section through the cloaca 40 μ cranial to the entrance of the pronephric ducts. 9 days previously embryo stained with Nile blue sulphate as indicated in Text-fig. 5. Nile blue sulphate seen as granules in the cloacal wall (*c*) and in the right pronephric duct (*p*). × 300.

Fig. 1.

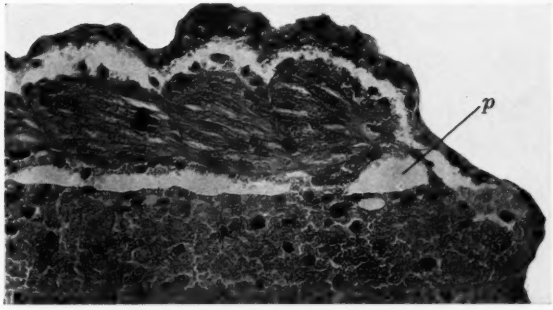

Fig. 3.

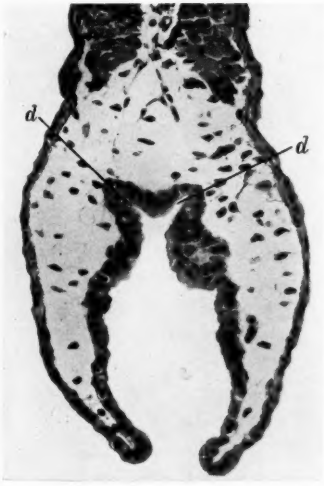

Fig. 2.

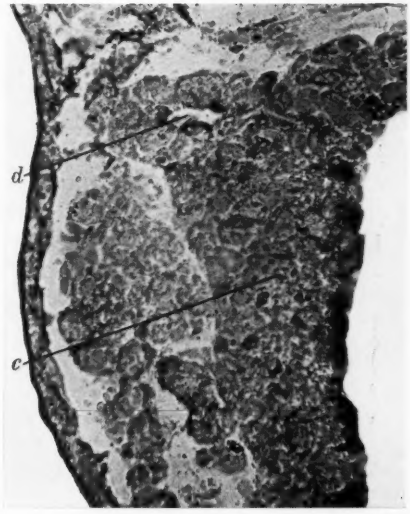

Fig. 4.

UNIV. OF MICH.

STUDIES OF THE REGENERATION OF LYMPHATIC VESSELS

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INTRODUCTION

The following paper describes in sections work which has been going on for three years on some of the problems arising in connexion with the regenerative growth of lymphatic vessels in human and animal tissues.

In the proximate analysis one may distinguish two groups of problems in this field, the first concerned with the stimuli adequate to cause regenerative growth of the vessels and with the nature of this initial growth, and the second with the factors responsible for the subsequent elaboration of the vessel walls, that is, orientation, modelling, and valvular development in any of the newly formed lymphatics. While following the phenomena of regeneration in the living animal it will be seen that these two main problems overlap to some extent, but, broadly speaking, attempts have been made to analyse first the factors concerned in regeneration in the adult of the smallest lymphatic capillaries, next the capacity for regenerative growth of the larger lymphatic trunks in the adult, and lastly the laws governing the reformation in the adult of a fully formed system of functioning vessels orientated and valved for the carriage of lymph in an appropriate manner.

In the analysis of this series of interrelated problems it is difficult to set out experiments in a perfectly simple succession following logically from one deduction to the next. Also in the course of three years of work the technique and methods at one's disposal develop appreciably, so that later work yields more precise information than the earlier. For these reasons it is proposed to set out the work in the order in which it was actually done irrespective of the logical sequence of conclusions which appears from the whole, the expression of which is left to the summary. Relevant literature also is considered separately in each of the three sections of the work.

The first section deals with observations made from human material, and is subject to the serious limitations imposed by lack of proper control tests and the difficulties of complete analysis. In spite of this it is felt that the observations are worth recording. The second and third sections of the work were done in the two succeeding years, the second consisting of experiments on animals and yielding information chiefly of a preliminary and elementary character, while the third, which also describes experiments on animals, goes further into the details of the problems outlined above.

PART I. REGENERATION OF LYMPHATIC VESSELS IN HUMAN TISSUE

Literature

Although it must have been evident for a very long time that at least in some circumstances re-establishment of lymphatic circulation after incisions into the tissues must occur, there is only a relatively small literature dealing with this subject. Taking the significant papers more or less chronologically, it will be seen that Billroth (1863) failed to find that any lymphatic vessels developed across scar tissue in the early stages up to 7 days. On a different aspect of this same fundamental problem of the initial causes of lymphatic regeneration, Krause (1863) reported the growth of new lymphatics into tumour tissue. Then came a series of papers by Bayer (1885), Delius (1888), Ribbert (1889), and Heuter (1904) towards the end of the century, describing the rather equivocal results of lymph-glandular regeneration following excision. Unfortunately, it is not clear from these papers what changes, if any, were observed in the lymphatic vessels. The next literature to be noticed consists of quotations by Bartels (1913) that Talke found new lymphatics in pleuritic membranes, and Poirier and Morau found new lymphatics in recto-uterine adhesions.

These general statements of the previous century were followed by Meyer's work (1906) on the extirpation of lymph glands. Among other experiments he tied and divided lymphatics around the saphenous vein in eight dogs, and observed the animals for periods of 14–91 days. He could find no evidence of regeneration of these vessels, and attributed the failure to insufficient lymph stasis.

T. H. Coffin (1906) found new lymphatics growing in granulation tissue 10 days after bringing a loop of intestine outside the abdominal wall. H. M. Evans (1908) showed the presence of new lymphatic vessels in relation to a sarcoma deposit. Eloesser (1923) experimented on obstruction to lymphatics due to scar tissue by making incisions across the dorsum of rabbits' ears down to the cartilage, and leaving them to heal by granulation. On injection of the lymphatics with India ink it was found that only a few regenerated vessels existed across the wound, and the scar obstruction to the vessels persisted in animals examined as long as 4 months after the operation. A more scientific analysis of the effects of severing lymphatic vessels was carried out by Reichert (1926), and more recently the problems have been touched upon by the researches of Rouvière & Valette (1937). These latter papers, and the work done by Pullinger & Florey (1937), will be considered more fully in other parts of this paper.

The human material examined, methods, and summary of results

The human material used came from a variety of sources. In fact, any human material which might shed light on the problems was used, and I have to thank the surgeons of St Bartholomew's Hospital, and particularly Sir

Harold Gillies, for the generous way in which they gave me every possible help and facility.

The tissues so examined were either living or freshly removed at operation and still warm when they reached the laboratory. In each case the lymphatic vessels of the tissue were injected with thorotrast by the method of Woollard & Gray, which was first reported to the Anatomical Society in 1935, and later described fully by Gray (1939). X-ray photographs of the tissues were taken, and where possible the specimens were subsequently dehydrated and cleared by the Spalteholz method. Thick sections, as well as thin stained sections, were examined microscopically.

It may be stated before describing the results that the only control which can be used in examining adult human lymphatic vessels in this way is supplied by the fact that all authoritative accounts of the normal human subcutaneous lymphatic trunks agree in claiming a relatively high constancy of topography, involving position, direction and even to some extent number of these vessels in the different regions of the body. Study of the works of such authors as Sappey (1874), Poirier *et al.* (1903) and Rouvière (1932) reveals this, and my own injections of the normal have confirmed it.

Human tissue type no. 1. This was a specimen of the skin of the leg distal to an epithelioma which was circumferential in growth about the ankle, and had involved the skin of the leg in front and at the sides of the ankle, leaving only a narrow wick of normal skin posteriorly to drain the lymph from the whole foot. The ulcer was of varicose origin, subsequently becoming malignant, and causing deposits in the popliteal and inguinal lymph glands. In the areas affected there was much destruction of underlying tissue, exposing even the periosteum in patches. This tissue, and the findings obtained, have already been described in another paper (Gray, 1939) as tissue no. 9. At the margins of the ulcer in certain regions there was the usual warty overgrowth of the dermal papillae. This was so extensive that some individual papillae had attained a length and breadth of several millimetres. In such papillae very extensive networks of lymphatic capillaries were found. This observation shows that severing of a lymphatic capillary is not the only and essential stimulus necessary for the growth of these vessels in the adult. Also from a study of these enlarged papillae some indirect evidence is obtained as to the method by which this new production of lymphatic vessels occurs. Theoretically, there are several possible ways in which such a network of vessels could be formed. That they might be produced out of non-lymphatic tissue independently of pre-existing lymphatic vessels can be excluded finally because of the fundamental researches on the subject by Sabin (1902) and E. L. & E. R. Clark (1932). But from pre-existing lymphatics in the dermis they might be produced either by interstitial elongation of the loops and segments of the network, or by a sprouting of new segments as blind diverticula which later join other segments to form new loops. Lastly, both of the latter phenomena may occur together. The density of the network in these enlarged papillae

does not favour the first of these latter possibilities as the sole method. Also examination revealed that in addition to the closed network in the papillae there were a number of blindly ending diverticula projecting from the network particularly at its peripheral margins, and showing the apex or free end of the tube in most cases turned back like a hook. The picture was thus suggestive of a sprout not yet united completely to form a loop.

The above account describes the findings in the first type of human tissue examined in their relation to the first group of problems of lymphatic regeneration outlined in the introduction.

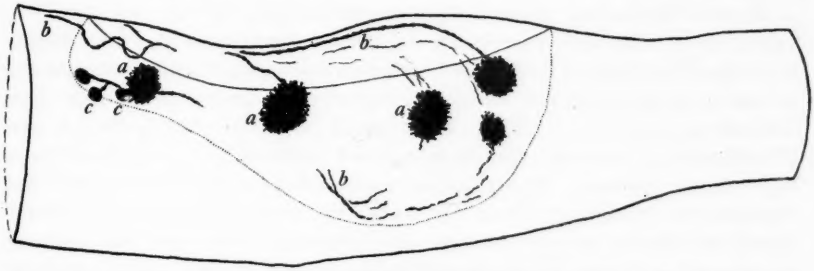
This tissue also provided some evidence in relation to the problems of vessel remodelling and reorientation as seen in the adult. The skin of the whole leg was removed in one piece, and later divided into many portions for detailed study. A plan of the whole skin was drawn, and the course which would be taken normally by the subcutaneous lymphatic trunks of the leg compared with that actually obtaining in the specimen. The findings have been recorded incidentally in a previous paper (Gray, 1939), to which reference must here be made. They show that in the pieces labelled "*E*" and "*G*" in the figures, representing skin overlying and postero-inferior to the lateral malleolus, the lymphatic trunks have changed their direction by about 90° to run backwards and upwards into the narrow area of normal skin overlying the tendo Achillis.

The presumption is that this change from the normal has occurred by the enlargement and further development of small existing anastomotic lymphatic vessels in response to the damming of the lymph flow by the extensive ulcer. Of course the findings provide no proof of this, but, as they stand, they are a definite contrast to the normal arrangement of lymphatics for this region. It may be added that at the time of operation there was considerable lymphoedema of the foot, and the specimen after injection of the lymphatics with thorotrast shows considerable dilatation of the lymphatic vessels below the level of the ulcer, but no noticeable dilatation above the ulcer. The very great number of medium-sized lymphatic trunks seen in the area of the narrow "wick" of normal skin overlying the tendo Achillis (piece *F*, Figs. 333, 336, Gray (1939)) is very different from the normal found for this region. Whether it is due to a dilatation of vessels or a growth of new channels cannot be determined from the specimen, though the former explanation seems the more probable.

Human tissue type no. 2. This tissue was studied in the living subject. Injections of the lymphatics with thorotrast were done in a large skin graft, which had been transplanted whole as a vascularized flap from the anterior abdominal wall to the volar surface of the right arm and forearm. The patient, Miss C. T., aged 28 years, was under the care of Sir Harold Gillies at St Bartholomew's Hospital, and was treated by plastic operations for the relief of lymphoedema of the legs. The history of the case and the successful operations devised have been published by Gillies & Fraser (1935). For the purposes of the present paper it may be stated that the transplanted skin came from the right

side of the anterior abdominal wall, and included areas in which some lymphatics ascend towards the axillary glands while others descend towards the inguinal glands. The graft was transplanted on to the volar surface and medial side of the right arm and forearm in such a way that there could have been no possibility of accurate alignment of the lymphatic trunks already in the flap with those in the arm skin around the edges of the flap. The graft had been united for many weeks, and there was no oedema of the flap at the time of injection.

The findings are indicated in Text-fig. 1, from which it will be seen that they have a bearing on the second group of problems of this work, the final modelling of regenerated lymphatic vessels. The final vessels resulting in the transplanted skin do not in any way correspond in pattern or direction with those of abdominal skin. But they do follow roughly the lines which vessels would take in normal arm and forearm skin in this situation. Some of them



Text-fig. 1. Diagram constructed from radiographs of right upper extremity of a patient with abdominal skin transplanted to medial side of arm and forearm. Thorotrast injections (*a*) were made at five areas within the transplant, whose limits are indicated. Lymphatics are shown as wavy lines (*b*). Supratrochlear glands have been filled (*c*).

have succeeded in establishing connexion with the supratrochlear lymph glands.

As in the first type of human tissue examined it is seen that the final result of the remodelling and reorientation phenomena seems to accord with the particular functional requirements of the case, and one is inclined to attribute the changes to "hydrodynamic" forces which may act in a way analogous to Thoma's first and second laws for blood vessels.

Human tissue type no. 3. The third type of human tissue also has some bearing on the second group of problems. This tissue was a portion of a tubed pedicle made from the skin of the iliac region of the anterior abdominal wall for use in the cure of hypospadias. I was indebted for this specimen also to Sir Harold Gillies. The pedicle was constructed by the usual method of making two parallel incisions followed by turning under and suturing together the two edges of the strip of skin thus freed. The length of the pedicle given to me was 6·5 cm., and this portion was taken from the lower and medial part of the entire

structure, which lay parallel to the inguinal ligament and was about 15–18 cm. in total length. The pedicle had been left for some 3 weeks before the specimen was removed.

Many injections of the specimen were done, and the findings, deduced from the radiograph and from thick cleared sections and thin microscopic sections, indicate that there have certainly been some changes leading to a rearrangement of the lymphatic trunks in the course of 3 weeks. The normal arrangement of the lymphatics in the skin used for construction of the pedicle is preserved as a series of loops of vessels circling round in the fat of the pedicle in a plane at right angles to its long axis (Pl. I, fig. 1). The chief modification which has occurred is that there is seen a tendency for these loops to become transformed into vessels running parallel to the long axis of the pedicle. The change is most noticeable among the smaller vessels visible in the radiograph. The larger trunks have retained the encircling disposition they were given when the pedicle was made.

It may be supposed that the small vessels tending to run parallel to the long axis of the pedicle represent pre-existing anastomotic channels, which, because of the necessity for conducting lymph one way or another out through an end of the pedicle, have become enlarged in the course of 3 weeks. Unfortunately, no specimen of a similarly placed pedicle left *in situ* for a longer period has been available. Although caution is necessary in making deductions from a single specimen, it may be said that 3 weeks is not sufficient time for very marked changes in the larger lymphatic trunks in response to special stimuli of altered flow and pressure, but is probably adequate for some readjustment sufficient in the circumstances of the present case to prevent lymphoedema.

The readjustment which has occurred may have taken place in one or more of several possible ways, which cannot be further elucidated from this single specimen. There may have been a dilatation of existing anastomotic channels as mentioned above; there may have been a growth of new capillary lymphatic vessels which became later elaborated into small trunks; or, lastly, some trunks themselves may have sprouted new vessels either along their length or at their ends severed when the pedicle was made.

PART II. REGENERATIVE CAPACITY OF LYMPHATIC VESSELS

Literature

Literature describing experimental work on this subject is not very extensive. The work of Meyer (1906), of Coffin (1906) and of Eloesser (1923) has been mentioned already. From such papers it appears that, although regenerative growth may occur from capillary lymphatics, very little, if any, can be expected from definitive lymphatic trunks. The reasons presumed by the authors for this limitation are either difficulty in producing sufficient lymph-

stasis pressure in the severed trunks, or interference by scar tissue. Substantial evidence for the first of these suppositions is not obtainable from Meyer's work, and full histological analysis of the alleged interference by scar tissue is lacking in Eloesser's work.

In 1926 Reichert carried out an accurate analysis of the results of severing lymphatic trunks followed by replacement of the cut ends. After many trials he perfected a technique for making a circular incision through the middle third of the thigh leaving only the femur and femoral artery and vein. These too were stripped of all areolar tissue, and the incision went through the periosteum and the adventitia of the vessels. It was followed by very careful resuture of all the tissues. Healing by first intention with the minimum of scar resulted. Lymphatics were then injected with India ink in animals killed at intervals of 2 days to 14 months after the operation. The results are worth quoting in detail, as they not only represent a definite advance in knowledge but serve as the starting point for further work.

When the operation was perfectly done, Reichert found that the earliest regeneration appeared in the superficial set of lymphatics 4 days after the operation. At three or four areas along the line of skin division tiny lymphatics (presumably as seen with the naked eye) ran from the distal vessels across the cut, and then converged again to enter a single lymphatic on the proximal side. Elsewhere superficial lymphatics only filled with ink up to the cut. If much scar was formed, no such union of vessels was found to occur. Regeneration of deep vessels was seen first at 8 days, vessels which twined about in relation to the muscle septal sheaths crossing the wound as minute segments. From later injections, up to 20 weeks, it was seen that the main trunks were often united, sometimes with no evidence of the cut, sometimes with a kinking or irregularity in the vessel at the point of cutting. To be correlated with these findings there was oedema distal to the cut, beginning on the second day, reaching maximum on the fourth or beginning of the fifth day, and disappearing completely by the eighth day. Reichert summarized his results in the observation that there was some arterial regeneration by the third day, and some venous and lymphatic regeneration by the fourth day. He also tried to find out the relative importance of the veins and the lymphatics for the relief of the oedema by ligaturing the femoral vein at different times after severing the lymphatics. Ligature of the vein at the time of maximum oedema caused only slight delay in its subsidence. Ligature at 8 days caused reappearance of oedema only if the lymphatics were blocked by India ink. Ligature after longer intervals caused no oedema even if the lymphatics were also blocked. From this he concluded that the lymphatics are important in relieving oedema at the 8-day stage. Also from these experiments he reached the conclusion that lymphatico-venous stasis is a stimulus for the regeneration of both deep and superficial lymphatics. Reichert's experiments do not decide whether it was the severing alone, or the lymphatico-venous stasis alone, or both of these, which stimulated the regeneration. His account was confined, as far as can

be determined, to naked-eye appearances. But his experiments do show that the cut ends of lymphatic trunks can rejoin to re-establish the circulation, if they are placed in apposition and the conditions as regards scar tissue are favourable. The exact methods and steps by which this occurs were not examined.

Analysis of the exact method of growth and regeneration of the lymphatic vessels has been done chiefly by American anatomists. Sabin (1902) proved that in the embryo all lymphatic vessels arise by sprouting from pre-existing capillary lymphatics, and E. L. & E. R. Clark (1932) followed the growth phenomena of lymphatic capillaries in the living adult rabbit's ear. They extended Sabin's statement to include new lymphatic vessel growth in the adult, and they were able to watch isolated capillary lymphatics for long periods of time. It is to be noted that they only recorded sprouting from the fine vessels. The large valved trunks with composite walls are not given such capacity by these authors. In fact in one of their papers they describe how the cut ends of large lymphatic trunks remain open in direct connexion with the tissue spaces for long intervals of time. It is to be noted that this observation was made on lymphatic trunks which had been torn or severed in the field of the transparent chambers used by these workers in the rabbit's ear. The environment of such vessels was therefore perhaps not absolutely identical with that normally existing.

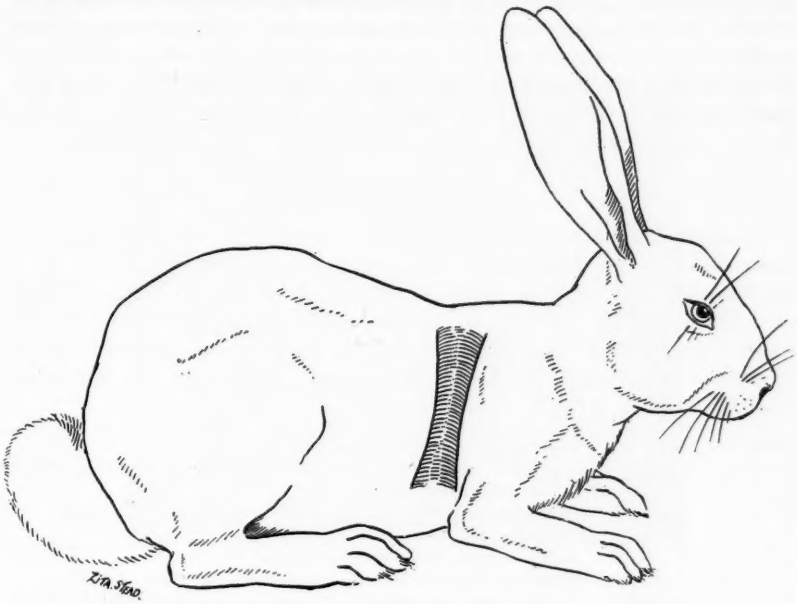
The transparent chamber studies of E. L. & E. R. Clark have also shown in precise detail the manner in which fibrous connective tissue interferes with the growth and form of lymphatic capillaries. But the most striking illustrations of this mechanical influence of fibrous tissue were obtained by Pullinger & Florey (1937), who gave pictures of the rich network of minute lymphatics which invade an inflamed area at the early stages, only to be driven back by the subsequently elaborated scar tissue, until finally the central area is quite devoid of these vessels.

Material and methods

Attempts were made to determine the regenerative capacity of lymphatic capillaries and particularly of the trunks, and if possible to throw light on the second group of problems of this work by experiments on rabbits. The procedure was to watch certain selected lymphatic vessels over a period of time after application of the stimuli necessary for their growth. For purposes of identification it was thought necessary to isolate a group of lymphatics and subject them to the same treatment. This was achieved by constructing tubed pedicles in the skin of the flank of adult rabbits as shown in Text-fig. 2. The usual method of construction employed in surgery was adopted, anaesthesia was produced by ether, and full asepsis was adhered to throughout. The pedicles were left for some weeks before further operations were attempted.

The number, position, direction and size of the lymphatic trunks in the pedicles were estimated by radiographs taken after thorotrast injection into

the skin of the flank just above the pedicle. With pedicles 2–3 inches long it was found very convenient to use dental X-ray films placed between the pedicle and the body of the animal. Normality of lymph flow in the pedicles was also determined before proceeding further by noting whether all thorotrast disappeared from the vessels with normal rapidity. Specimens in which the lymphatic trunks did not run parallel through the length of the pedicle, or in which the rate of flow of the lymph was for some reason or other not normal, were rejected as unsuitable for further work.



Text-fig. 2. Indicates position and extent of tubed pedicles constructed in the flank skin of rabbits.

A second operation was then performed. In this the pedicle was divided transversely across its whole width, and the cut surfaces then immediately resutured as carefully as possible. In some instances a small sample (3–4 mm. in length) of the pedicle was removed during this operation. The sample which was removed from one cut surface of the pedicle provided a picture of all the lymphatic trunks running along the pedicle, its width being the same as that of the pedicle. Transverse sections of the sample were cut and stained.

The fate of the severed lymphatic trunks in the reunited pedicle was then watched from radiographs after thorotrast injection at intervals. Animals were killed after varying intervals, and at the time of death a last thorotrast injection was made. The pedicle was cleared by the Spalteholz method for

examination in its entirety under low magnification, and in thin sections cut from paraffin blocks for miscroscopic examination.

Modifications of these experiments were tried in which a cut end of one pedicle was implanted into the ordinary skin of the flank, or the cut end of a pedicle was sutured to a freshly incised free end of a previously constructed pedicle stump.

It was hoped that these modifications would not only serve as checks on the main experiments, but also throw light on the influence of lymph-flow pressure upon the phenomena of growth, anastomosis and remodelling of the vessels. Unfortunately there were not enough successful instances of these modified operations to allow of any deductions. The few in which union of the pedicle stumps occurred showed such large amounts of scar tissue that lymphatic growth or anastomosis was completely interfered with. It may be that further trials of these modified operations will later be more successful.

Results of Experiments

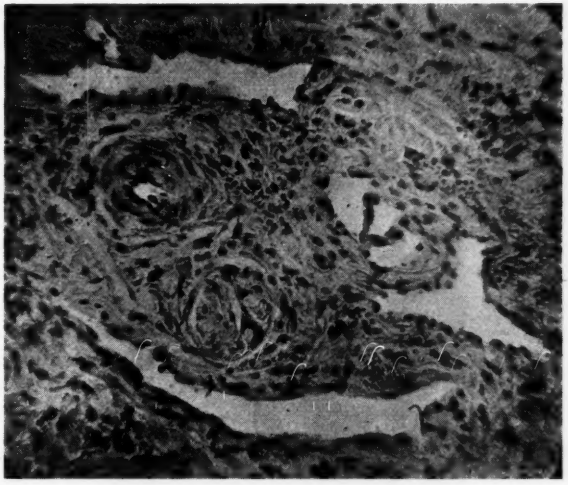
Suitable pedicles were made in twelve adult rabbits. Of these four were successfully severed and reunited under ether anaesthesia, and the events in the lymphatic trunks followed with thorotrast. The relatively high percentage of failures was due to the considerable technical difficulties involved. The successive operations and tests to which the animals were subjected can be seen from the protocol for one of the animals, which may be regarded as typical.

Rabbit 4	23. iii. 38	Pedicle made
	22. iv. 38	Thorotrast injection of flank skin above pedicle; X-ray of pedicle (30 days after construction)
	10. v. 38	Pedicle severed, and cut ends rejoined after removal of a sample.
	18. v. 38	Thorotrast injection and X-ray as before.
	27. v. 38	X-ray of pedicle without further injection.
	1. vi. 38	Thorotrast injection and X-ray as before.
	8. vi. 38	Animal killed; thorotrast injection and X-ray of pedicle taken at death. Pedicle then cleared for examination and section, 27 days after severing and reunion.

The findings in the successful operations were consistent with one another, and a number of points emerge which have not been demonstrated in the literature on the fate of severed lymphatic trunks.

First of all attention may be called to the appearance of the lymphatic trunks when injected with thorotrast in the pedicle as yet unsevered. The ordinary appearance is shown in Pl. I, fig. 2. Three trunks are seen definitely as fine lines running along the pedicle. The direction of flow of lymph in all these pedicles was from dorsal to ventral. As an important contrast with this appearance of normal undilated trunks in the pedicles, one may examine Pl. I, fig. 3, which shows the effect of compression of the lower end of a pedicle during thorotrast injection. The figures give an idea of the degree of dilatation of lymphatic trunks which can occur, and this should be borne in mind when

considering the later figures in this section. A striking point also to be noted is the aggregation of thorotrast near the walls of the vessels giving the appearance of two parallel lines to be seen in the dilated vessels. This finding is no isolated one, but its explanation is not clear. It may be associated in some way with the well-recognized capacity of lymphatic endothelium for a kind of athrocytic absorptive activity towards particles of certain dimensions. The particles of thorotrast with a molecular size of 80 A. may come within the range of size of particles for which athrocytosis[1] has been observed. On the other hand, it may be that there are purely mechanical reasons for a segregation of heavy thorium particles towards the walls of the vessels.



Text-fig. 3. Photomicrograph of 10μ transverse section of part of sample of a rabbit pedicle taken 2 days after the operation of severing and reunion. $\times$ 110.

In regard to the events in the lymphatic trunks after severing, it may first of all be stated that the cut ends of the trunks become sealed off within a day or two. These experiments do not exclude the possibility that the sealing off occurs within a matter of hours. Although the ends of the trunks are thus closed, there is no extensive thrombosis along the vessels. A striking contrast is presented with the small blood vessels. None of the pathological changes which occur in the walls of the blood vessels at their cut ends are to be seen in the lymphatics. One may account for these effects by postulating that the lymphatic trunk is sealed by a simple growth forwards of the severed endothelium of the tube, the actual closure being perhaps brought about by the mechanical guiding influence of the surrounding fibrous connective tissue.

[1] The term "athrocytosis" is used to designate the intracellular flocculation of certain colloidal particles (see P. Gérard, 1935–6).

Whatever the mechanism of closure, its occurrence is definitely shown in Pl. I, figs. 7–10. Text-fig. 3, which is a photomicrograph of a portion of a sample from a pedicle in an animal which died of intercurrent pulmonary disease 2 days after the operation of severing and reunion, shows how thrombotic changes are absent from the wall of the lymphatics while present in those of an artery a little distance above the level of severing. A few sections below the one illustrated, the little artery showed the early signs of organization of the clot in its lumen and an absence of endothelium. Though the lymphatics in the specimen were not injected, one can be quite certain that some of the dilated endothelial-lined spaces in the picture are lymphatics, and their obvious dilatation is to be contrasted with the state of the blood vessel.

Text-fig. 4. Photomicrograph of 10μ longitudinal section of upper part of a rabbit pedicle which had been injected with thorotrast after the operation of severing and reunion, re-injected 11 days later during life, and then killed and given a final injection after a further 8 days. Fresh (unphagocytosed) and old (phagocytosed) thorotrast can be seen in the same lymphatic vessels. ×110.

The lymphatic trunks on the distal side of the cut (in the dorsal part of the pedicle) become considerably dilated, and their contents stagnate within the lumen. This is shown in Pl. I, figs. 7–10. No coagulation of the lymph occurs in the lumen. The fluidity of the contents for weeks after the end of a lymphatic vessel is sealed has been demonstrated by the work of E. L. & E. R. Clark, and is here suggested for the pedicle lymphatics by varying amounts of thorotrast in them at different injections made at intervals after the severing (see Pl. I, figs. 7–10). It is finally established by examination of microscopic sections of pedicles made after "follow-up" injections of thorotrast up to the time of death. From these (Text-fig. 4) it is seen that thorotrast which has been

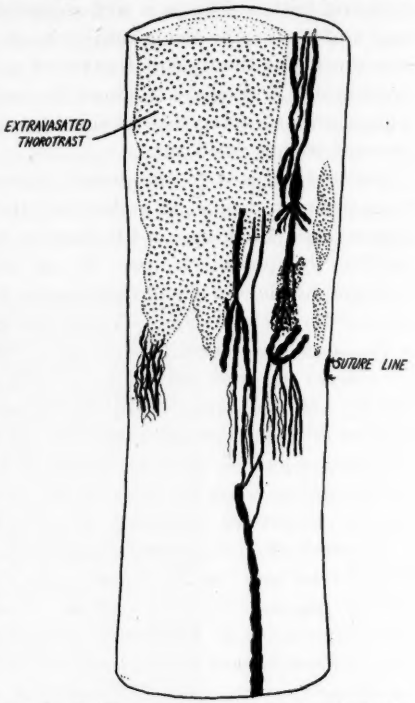
injected into the lymphatics some time previously has been ingested by phagocytes, which have wandered into the lumen of the lymphatic from the surrounding tissues. This is a well-recognized phenomenon, which has actually been watched in the living subject by E. L. & E. R. Clark. Thorotrast which was injected at the time of death is of course not ingested in this manner. An examination of Text-fig. 4 shows the two lots of thorotrast, from earlier and later injections, lying side by side in some of the vessels. Coagulation of the contents is thus ruled out.

Next, in regard to the growth phenomena shown by the lymphatics in these pedicles, it must be stated that the radiographs show only macroscopic appearances (Pl. I, figs. 4–10), and can therefore give no direct indication of capillary lymphatic changes. At an interval of 8 days after the severing, radiographs showed no obvious connexion of lymphatic trunks on the two sides of the incision. In X-rays of one specimen (Pl. I, fig. 7) there are faint indications that thorotrast is being conducted across the scar, but it is not possible to say from the picture whether an anastomosis of trunks exists, or exactly what the nature of the connecting vessels is. By the time 3 weeks have elapsed after the operation, radiographs present no doubt that the trunks in the upper segments of the pedicle have become anastomosed with those in the lower segment below the incision. At this stage there may still be only a very narrow constricted connexion between the two portions of the vessels, and some trunks do not appear to have been successful in establishing connexion. But at least some have got connexions large enough to allow sufficient thorotrast to pass into the lower lymphatic trunk to render it opaque to the X-rays. The claim made in Reichert's work that lymphatic trunks can become reunited when severed is thus confirmed. The longer time taken for union in my experiments is not in conflict with Reichert's findings, because in the pedicle experiments there was no attempt to get exact apposition of particular severed trunks, and scar tissue formation was probably more abundant in my experiments.

It may be recorded that some oedema occurred in the upper segment of these pedicles when they were severed, and its duration corresponded roughly with that found by Reichert after incisions through the whole lower limb. But for obvious reasons my experiments, as carried out, were not suitable for studies of oedema in the same way as were Reichert's operations in which drainage from a whole limb was interfered with.

The work was carried further by the findings obtained from cleared pedicles, and from microscopic examination of these. Text-fig. 5 is a hand drawing of a cleared specimen obtained from the same pedicle as is illustrated in Pl. I, figs. 4–10. The cleared specimen was split in halves longitudinally, and the diagram shows the appearance of one of these halves when examined in oil of wintergreen under ordinary binocular magnification. The stumps of severed lymphatics are seen, and uniting these are a number of fine vessels which must have sprouted out from the trunks, grown along the pedicle parallel to its length,

and finally established connexion with stumps of trunks on the other side of the incision. Particularly striking is the growth of a sort of spray of fine lymphatic vessels from the region of a valve in one trunk some distance away from the cut end of the vessel. To my knowledge this kind of sprouting has not been recorded hitherto. The sprouts from the cut ends of the vessels may have owed their origin to the single stimulus of the severing of the endothelium, or to the influence of the pressure of lymph stasis in the vessel, or to a combination of both forces. But the "spray of sprouts" from a region several millimetres above the stump of a severed trunk must be attributed to lymph pressure, and cannot be caused by any "setting free" of endothelial cells by cutting.



Text-fig. 5. Diagram of some of the lymphatics in a rabbit pedicle 27 days after severing and reunion operation. Stumps of lymphatic trunks and sprouts from these are shown effecting reconnexion.

The vessels uniting the trunks across the scar are narrow and very thin-walled. There has not been developed any assemblage of muscle and adventitial layers around the endothelial walls of these vessels in the course of the period of about one month for which they were observed.

From the above experiments it will be seen that the main results of Reichert's work are confirmed, and details of the steps in the phenomena he observed are now displayed. More definite evidence is presented that lymph stasis can cause sprouting from lymphatic trunks, although it is to be noted that the experiments do not exclude the possibility that the mere cutting of trunk endothelium is sufficient to start the growth of sprouts.

PART III. FURTHER EXPERIMENTS TESTING THE CAPACITY FOR LYMPHATIC VESSEL REGENERATION

Literature

It will be seen from the accounts of the literature and findings in the first and second parts of this paper, that besides the separate problems of the initial growth of regenerating lymphatics and the subsequent elaboration of newly

formed vessels, one must bear in mind that these two aspects of the subject may be grouped as one, both being concerned with factors intrinsic in the vessels themselves. Thus they may be contrasted with factors extrinsic to the vessels and yet affecting regeneration.

Recent studies, particularly those of Clark & Clark (1937) and of Pullinger & Florey (1937), have cleared up very completely the subject of the effects of fibrous or scar tissue upon lymphatic vessel regeneration. The mechanical effect of this "extrinsic" factor is now fully understood, and from such studies Clark & Clark have been able to explain the topographical association of lymphatic vessels with arteries and veins. Also, Pullinger & Florey have shown how inflammatory foci exert a stimulating influence on lymphatic capillary growth in the stages of acute inflammation associated with hyperaemia and early fibroblast proliferation.

The present section is concerned chiefly with the analysis of factors intrinsic in the lymphatic vessels themselves.

Rouvière & Valette (1937) have described the results they obtained from experiments in which they interfered with the circulation of lymph in the rabbit's hindleg by removal of the popliteal lymph gland. These authors give the literature references to other similar experiments, such as removing an area of the lymphatics at the base of the ear to note whether any re-establishment of the lymphatic circulation occurs. The final new courses taken by the lymph after such operations were figured, and it appears from such work that these new pathways are provided most often by a dilatation and elaboration of pre-existing anastomotic vessels. In some cases the fresh growth of some capillary lymphatics for very short distances was apparently necessary in order to effect the establishment of lymphatic vascular anastomosis. No further details about the actual steps of these processes are available. The same may be said of the findings by Ssysganow (1931) and those by Vonwiller & Vannotti (1931).

Material and methods

It was apparent that the best way of making further advance would be to operate upon a single lymphatic trunk, severing it from its connexions or transplanting it in some way to test its capacity for growth. For this purpose the rabbit's ear is a suitable region, because a considerable number of relatively large lymphatic trunks run fairly straight courses from the distal towards the basal part of the ear under the dorsal skin, lying in the loose areolar tissue outside the perichondrium of the ear cartilage.

Operations were performed with full surgical aseptic precautions under nembutal or ether anaesthesia, and a suitable combination of operation table, microscope stage, and binocular microscope was used.

Ophthalmological instruments such as iridectomy scissors, paracentesis needles and finely pointed forceps, proved the best instruments. Thorotrast was not a suitable injection material for these operations as it is not visible in the living tissues apart from radiography. For this purpose it was found that

ordinary blue-black ink, after removal of coarse particles by centrifuging, and approximate correction of its tonicity and acidity, was very suitable for interstitial injection of the lymphatics. At first the operations were all carried out on vessels so injected, and follow-up injections were done at intervals up to and including the time of killing the animal. But, in view of the possible irritating or toxic effect of the ink on the endothelium of the lymphatics, the same experiments were subsequently all repeated in animals in which no injection was done until the time of killing.

The individual vessels were reached by reflecting a rectangular flap of skin, and then working with knife and forceps under the binocular, the field of operation being kept moist by a saline drip. The skin flap was then replaced and its margins carefully sutured in place. Over twenty-five successful operations were carried out on ink-injected vessels besides many control and follow-up injections, and there were another ten operations on vessels which were not injected until the time of death. The operations may be classified as follows:

(1) Simple severing of a single lymphatic trunk. In some of these cases the trunk cut was one remote from other similar lymphatics or blood vessels. In others one member of the clump of trunks running with the main central blood vessels of the ear was severed. In yet other instances all members of this clump were severed. The severing was done usually by inserting the point of the fine sharp knife under the skin and cutting a particular vessel with the minimum of trauma. In some cases a particular vessel was severed after a skin flap had been reflected. The flap was then replaced and sutured. In one experiment done by chance, but which had interesting results, a trunk was severed in an area covered by a mica sheet fixed in position after the manner used in constructing a transparent chamber. The results were compared when the sheet of mica was left in place and when the sheet was later removed. This test was made on an uninjected trunk.

(2) Isolation of a portion of a lymphatic trunk 1–2 cm. long, by an enclosing rectangular incision made through the tissues under the skin. The vessel was thus left *in situ*, but cut off for a length from its connexions with other lymphatics. These experiments were devised in order to dissociate the influence of lymph pressure from that of severing upon lymphatic regeneration.

(3) and (4) Transplantation experiments in which a length of trunk was cut out from its environment and laid down in a new bed prepared for it in some other part of the ear. There were two types of this experiment. In one the transplant was orientated with its valves in the same direction as those of the trunks in apposition with its ends. In the other direction of the transplant was such that its valves were reversed relative to those of surrounding trunks. These experiments were devised to test the influence of the normal blood vessels of the lymphatic wall and its nerves upon regeneration.

(5) Removal experiments in which a centimetre or more of a lymphatic trunk was removed altogether. These experiments were planned in order to get a measure of the amount of growth possible from a severed lymphatic trunk.

At the end of the observation period for each animal an ink injection was made, the animal killed by overdose of anaesthetic, and the ears fixed and cleared by the Spalteholz method. The unoperated ear was a control for each animal and was likewise injected, although there is such a constancy of lymphatic trunk pattern in the ears of these rabbits, and the results of the operation were usually so definite, that this was scarcely necessary for every case.

The cleared specimens were examined after the cartilage and internal skin had been stripped off. In some cases a rough microdissection was carried out to determine the exact spatial relations of the vessels. When necessary, sections were cut from paraffin blocks and examined histologically.

Results of experiments

Before describing the results of the experiments it is right to notice the appearance of the vessels operated upon. A typical sample is shown in Pl. II, fig. 1. In this the valves and the interval between them can be readily appreciated. On histological examination with different stains such as haematoxylin and eosin, Passini's stain, Mallory's triple stain, and van Gieson, it was found that such a vessel, though possessing a relatively thin wall, yet showed a definite amount of smooth muscle tissue and a definite adventitia. The rich vascularization of the walls of such lymphatic trunks with blood capillaries has been pointed out by various authors, and was confirmed by Gray (1939, Fig. 316). They are also innervated by non-medullated nerves. In all the experiments the results were the same both when preliminary injections were made, and when no injections were made until the time of death.

(1) *Results of the simple severing of a lymphatic trunk.* Altogether twenty-six divisions of lymphatic trunks were completed. In twelve of these the vessels were definitely identified at all subsequent stages. Two of these twelve were operations performed with a mica window in place, and were not used for testing the uniting capacity of severed trunks. But these two cases do perhaps help to reconcile the conflicting claims of Clark & Clark (1937) that severed trunks remain open, and of Reichert and myself that they do not remain open, but first become sealed off and then later may join other vessels.

In one of these two cases a severed uninjected lymphatic was watched for a week through the mica covering. No sprouting could be seen under the binocular microscope, although when the right amount of transmitted and reflected light was used the vessel could be clearly seen. After a week the animal was anaesthetized and the mica sheet was removed. An appreciable amount of clear fluid lymph was found lying between the tissue and the mica. The vessel was then injected carefully with ink without causing any excessive pressure within it. The ink flowed freely out from the previously cut end of the vessel on to the surface of the connective tissue, which had been overlain by the mica. Further examination after killing the animal verified the open condition of the severed lymphatic.

It was thought that possibly the presence of the mica and a potential space between it and the cut end of the vessel had some effect upon the vessel, allowing perhaps a steady unrestricted flow of lymph through the open end, and thus preventing its closure. The operation was therefore repeated on a different animal and the mica sheet removed (under anaesthesia) at the end of 3 days. The same little pool of clear liquid lymph was found under the mica when it was removed. But 48 hr. after its removal the vessel end was found by injection to be closed, and a layer of fibrin containing leucocytes separated the stump of the vessel from the surface. Perhaps these tests need to be checked further, but at least they are suggestive.

Of the ten successful ordinary severings of trunks, four showed reunion of the cut ends, and union failed to occur in the rest (Pl. II, fig. 3). The minimum time for reunion in these experiments was 5 days. The sprouts from the cut ends were seen in one of the instances of union (Pl. II, fig. 2), and were also seen in three of the cases where union failed to occur. In one of the cases where union occurred it was found at the time of re-examination that a constriction was present at the point of incision. In another case, the vessel which united in 5 days, subsequent examination after an interval of 9 weeks showed that the original single reunited trunk had now split longitudinally into three small vessels at the area where the junction had been effected. The three parallel vessels then ran into one again a little below the point of the incision. This result is in keeping with the findings of Clark & Clark (1937) as an interference phenomenon due to coarse connective tissue fibres developing at the site of the original trauma.

These experiments leave no doubt that union can occur, and the steps by which it occurs are shown. But they also indicate the potency of fibrous tissue for interfering with the sprouts. It may be said that if the two ends of a severed trunk are more than about 1 mm. apart scar tissue will prevent their direct union.

(2) *Results of the isolation experiments.* Although this operation was tried in a number of animals, it was successfully followed up only in two. In one of these injections were made at intervals, in the other no injections were made at all until the animal was killed. The first animal was allowed to live for $10\frac{1}{2}$ weeks after operation, but the second was killed after 6 days.

The results showed that the isolated trunk or trunks remain intact, sprouts occur from them, and these join with sprouts from other vessels which have been cut but not isolated. The sprouts grow from both ends of the isolated vessel. The results are illustrated in Text-figs. 6–9, and Pl. III, figs. 1, 2.

The appearance of sprouts from both ends of the isolated vessels shows that the pressure of lymph within the vessels cannot be the one and only essential stimulus to their growth. At the distal end of such an isolated trunk there can be very little if any lymph pressure exerted on the interior. The valves remain intact in such a vessel, and must automatically prevent the transmission of pressure to the distal end of the cut vessel.

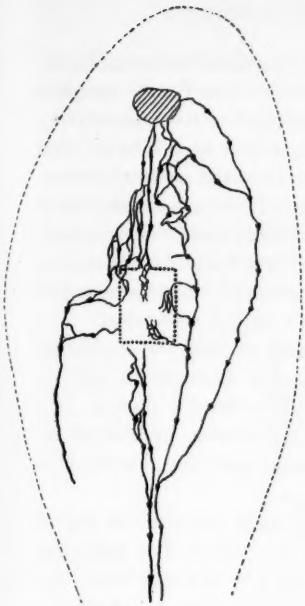


Fig. 6.

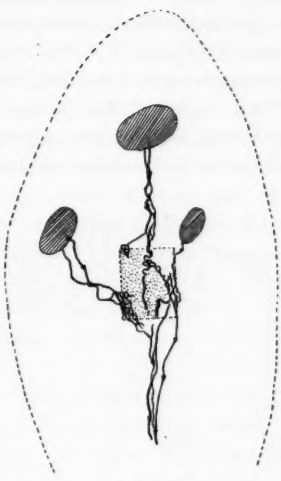


Fig. 7.

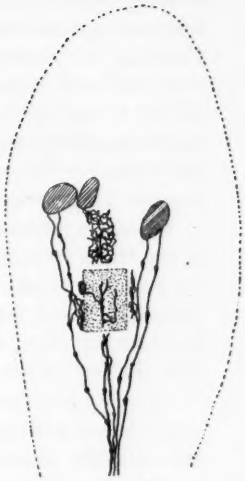


Fig. 8.

Text-fig. 6. Diagram of lymphatics in the ear of a rabbit in which isolation of the trunks was effected in the central area shown dotted. Injection 4 days after operation.
Text-fig. 7. As for Text-fig. 6. 4 weeks after operation.
Text-fig. 8. As for Text-fig. 6. 8 weeks after operation.

Text-fig. 9. As for Text-fig. 6. 10½ weeks after operation. Animal killed.

From the illustrations the influence of scar tissue can again be seen. In the case watched for 10½ weeks, Text-figs. 6–9, newly grown fine vessels are seen to have ranged themselves parallel to the strands of connective tissue, relatively few managing to cross the scar into the surrounding areas. It will be noticed also that the anastomoses established at the two ends of the isolated vessels were still only narrow vessels even after 10½ weeks. It may be that this is chiefly a scar tissue effect, and not due to any intrinsic incapacity of such anastomosing lymphatics to enlarge and strengthen their walls. The operative procedure for this experiment necessarily involved an appreciable amount of trauma to the tissue, so that scar formation was encouraged somewhat.

(3) *and* (4) *Results of the transplantation experiments.* In these the disruption of the trunk from its normal environment was obviously more severe than in the isolation experiments. Also, it was not possible to extract a portion of a lymphatic vessel without taking with it some of the surrounding areolar tissue, and this may have introduced an unwanted nutritional question in connexion with the fate of the transplant.

At first eleven transplantation operations, in which the vessel to be moved was injected with ink before being extracted, were performed. The graft was subsequently always identifiable in these cases by the phagocytosed ink particles in its lumen. As an additional aid to identification the graft was usually placed in its new bed with a double curve in its course. In two of the eleven cases the transplant was not reversed, and in nine it was reversed. In some of the cases the transplant was cut from among the central lymphatics, turned through 180°, and laid down again in the bed from which it had been taken. In others a length of a paramedian vessel was shifted into the central region. The grafts were all from 1 to 2 cm. long. In some cases the bed prepared for the transplant was scraped quite free of all tissue down to the cartilage, and when the graft was fitted in place it was covered only by the very thin dermis and epidermis of the skin flap. In other cases the bed was not cleared so completely of all other lymphatic trunks. In all cases the graft was placed as accurately as possible in apposition with the cut ends of the trunk or trunks at the proximal and distal ends of the bed. The grafts were watched for periods up to 19 days. Besides these eleven experiments, four similar experiments were made in which there were no injections at all until the time of killing the animal. These grafting experiments were difficult to carry out and bring to a successful conclusion, but certain fairly definite results emerge from them.

There was little evidence of sprouting from the grafted vessels. A few sprouts were seen from the ends of two of the grafts, but they were much less abundant than in the other types of operation. The graft in most cases seemed to remain passive without much evidence of growth or other changes in its walls (Text-fig. 11).

In all cases there was much growth of lymphatic sprouts from surrounding vessels into the region of the graft. Most of these sprouts failed to establish

connexion with the graft, but in some cases such anastomosis was definitely present. Text-figs. 10, 11 and Pl. III, fig. 3, illustrate this result.

It may be concluded that transplantation of a lymphatic trunk does not preclude its subsequent participation in the lymphatic circulation, though it does appear to interfere somewhat with its growth capacity. Unfortunately successful operations were not followed long enough to show the fate of the valves of the grafts in the reversal experiments.

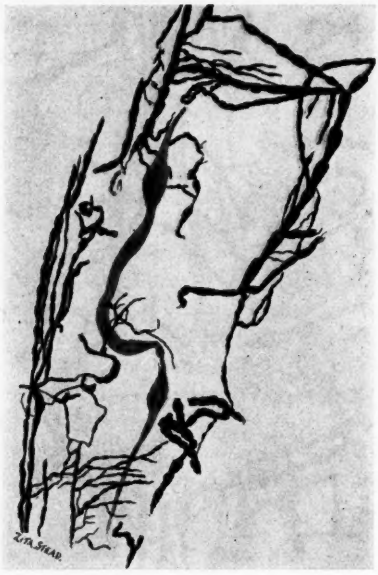
(5) *Results and findings from the trunk removal experiments.* Although many examples of removal of a length of lymphatic trunk (1–2 cm.) were

Text-fig. 10. Diagram of lymphatics in a rabbit's ear 13 days after transplantation of trunks (*a*) without reversal. No injections made until the time of death. Note impaired sprouting from the transplant and anastomosis of it with other trunks; (*b*) indicates areas where ink was extravasated at injection sites.

provided by the grafting experiments, only four operations were carried out specifically for the purpose of tracing the events following this procedure. In two of these four cases the removal may not have included all the lymphatic trunks in the area denuded. In the other two great care was taken to remove all trunks from the narrow rectangular field of operation. The animals were observed up to a maximum period of 16 weeks.

The follow-up and final injections in these cases showed only minute trunks just visible to the naked eye in the operation field some 14 days after operation. These were enlarged vessels which had not been removed at operation. The

sprouts from the severed vessels had only grown about a millimetre after 18 days, and this length was no more than was found in as short a time as 8 days. After a month, small vessels growing from the surrounding areas had joined up with these sprouts from the severed trunks, so as to vascularize most of the area of operation. But even after 16 weeks the normal lymphatic trunk pattern was not restored. No doubt the effects seen in this group of experiments are much influenced by scar tissue. But the conditions of operation in this group probably correspond much more closely to what happens to lymphatic trunks in ordinary surgical procedures than do the conditions in the

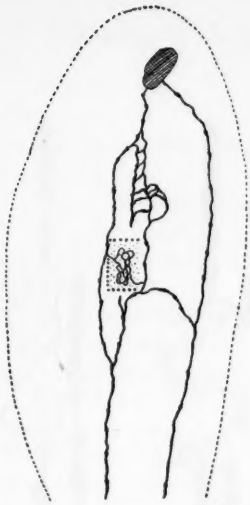


Text-fig. 11. Diagram of lymphatics in a rabbit's ear 12 days after transplantation with reversal of a single injected trunk. No sprouts from the transplant are seen, but connexion with sprouts from other vessels has occurred.

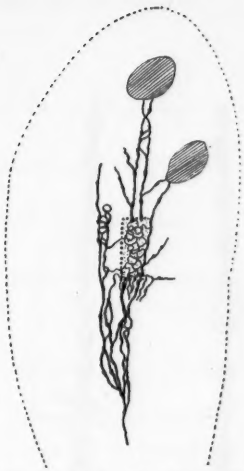
other groups of experiments. They show that in ordinary operative procedures growth from lymphatic trunks is much limited by scar tissue. But the great abundance of lymphatics in the regions where they are found at all will ensure re-establishment of the circulation in accordance with the descriptions given in this experimental work, even when relatively large areas have been interfered with. These findings are illustrated in Text-figs. 12–14.

Conclusions from operations on individual lymphatic trunks

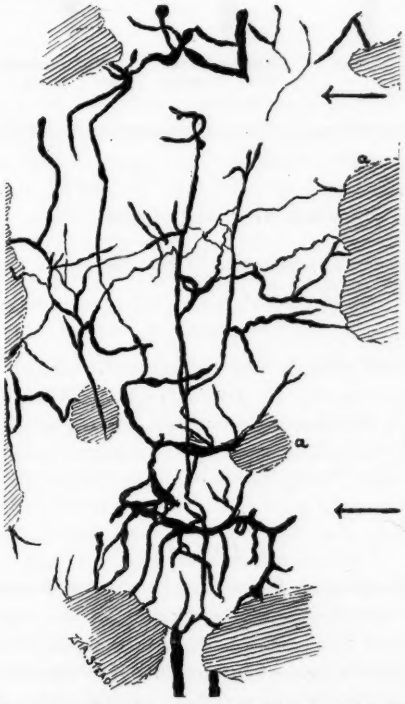
The main conclusions from the five groups of experiments which have been described above are epitomized in the diagram shown in Text-fig. 15.



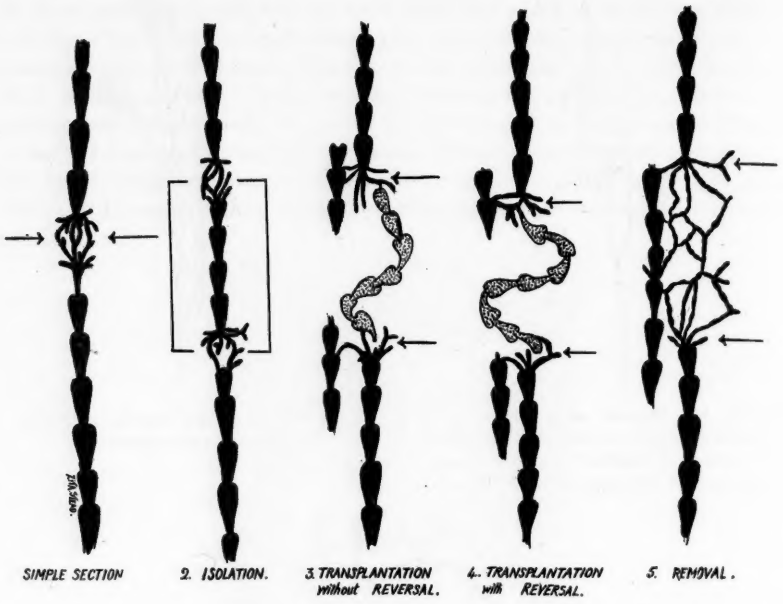
Text-fig. 12. Diagram of lymphatics in a rabbit's ear (R 17) 14 days after removal of about 1·5 cm. length of the central vessels. Fine vessels only are present in the area.



Text-fig. 13. As for Text-fig. 12 (R 17). 6 weeks after operation.



Text-fig. 14. As for Text-fig. 12 (R 17) at time of death, 16 weeks after operation. There is failure to restore the original lymphatic vessel pattern, although the flow of lymph is doubtless unimpeded. The limits of the trunk removal are shown by two arrows; (*a*) indicates extravasated ink.



Text-fig. 15. Diagram summarizing the results of the experiments on individual lymphatic trunks.

SUMMARY OF RESULTS

The following remarks summarize briefly the main findings from each of the three parts of this work.

Part I

Capillary lymphatic growth may be caused by stimuli other than cutting of the vessels. Hypertrophy of dermal papillae is such a stimulus. Very extensive remodelling and elaboration of pre-existing accessory or anastomotic lymphatic pathways may occur in response to altered pressure-flow conditions. Such remodelling is slow, being not very extensive in the course of 3 weeks.

Part II

Lymphatic trunks respond to severing in a peculiar manner quite different from that of blood vessels. There is sealing off of the cut but no thrombotic change. Sprouting and joining of trunks can occur after severing.

Sprouting from trunks caused by pressure-flow stimuli alone is seen.

Remodelling of newly formed vessels is influenced by fibrous scar tissue.

Part III

Severed trunks can unite, sprouting being the method, but scar tissue easily prevents this.

The sealing off of cut ends of trunks may require some stoppage or at least interference with lymph flow in the vessels.

A flow of lymph in a trunk is not necessary to the continued existence of such a vessel.

Sprouts from trunks may be caused by the stimulus of severing alone.

Sprouts from trunks may occur in response to the combined stimuli of severing and altered pressure-flow conditions.

Transplantation of a length of a lymphatic trunk does not preclude its subsequent usefulness as a conducting vessel for lymph.

Transplantation probably does impair the sprouting capacity of a trunk.

The total growth of sprouts from trunks is limited in practice by scar tissue, and by union with other vessels, to a few millimetres. This total is reached within 8 days.

The interference of fibrous scar tissue with the processes of remodelling and elaboration of new vessels in surgical procedures is very severe.

CONCLUSIONS

The work refers solely to adult mammalian lymphatic vessel regenerative phenomena. Human tissue subjected to pathological influences and to special surgical procedures has been examined. Experiments on lymphatics in tubed pedicles in rabbits and in rabbits' ears have been carried out.

Peculiar properties of lymphatic trunks. Thrombosis does not ordinarily follow trauma to trunks. A flow of lymph is not necessary to the continued existence of a trunk in the body. Tears or cuts in a trunk may remain open if the flow in the vessel is quite unrestricted. Tears or cuts in trunks do ordinarily become sealed up, and this may be associated with some damming up of the lymph flow. Transplantation of a trunk does not prevent its being used subsequently in the lymphatic circulation.

The initial regenerative growth of lymphatics in the adult. Such growth may occur from both capillaries and trunks. The method is by sprouts in both cases, and the sprouts may succeed in uniting trunk with trunk. The stimuli adequate to produce this growth may be cutting alone, altered pressure-flow conditions within the vessels alone, or a combination of these. Hypertrophy of dermal papillae is another adequate stimulus, but this has not been further analysed. Transplantation of trunks impairs their sprouting capacity. The total growth in length of sprouts as such is limited in life to 1–2 mm. by fibrous scar tissue or by union with other vessels.

Subsequent elaboration and modelling of lymphatic vessels. Remodelling and elaboration of pre-existing small vessels into large definitive trunks occur in

response to altered conditions of pressure and flow of lymph. These changes are very seriously hampered by fibrous connective tissue. Modelling and elaboration of newly formed lymphatic vessels occurs, but is in practice usually much slower, and also is much interfered with by scar tissue. The rate of these processes is to be measured in terms of weeks.

ACKNOWLEDGEMENTS

Some of the work of this paper was aided by grants from the St Bartholomew's Hospital Cancer Research Committee in 1935–6, and by a grant from the British Empire Cancer Campaign in 1937.

I am much indebted to the late Prof. H. H. Woollard for his interest in the work and his readiness to help in every possible way. Some of the methods used were worked out in collaboration with him, and his continual encouragement and research skill were always given to me freely and fully.

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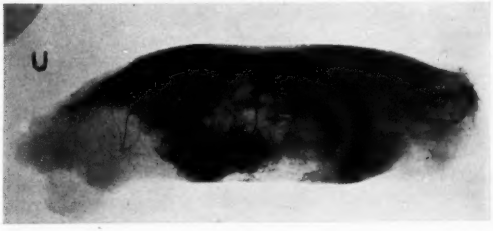
EXPLANATION OF PLATES I–III

PLATE I

All ten figures are of normal size, unmagnified

Fig. 1. Radiograph of portion of human tubed pedicle after injection of lymphatics with thorotrast. Upper lateral end of the specimen is shown by the letter *U*.

Fig. 2. Radiograph of a rabbit pedicle 2 min. after thorotrast injection of its lymphatics. Note the size of the vessels.



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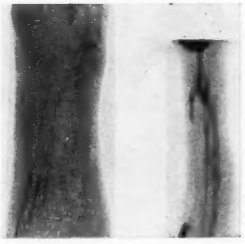

Fig. 2. Fig. 3.

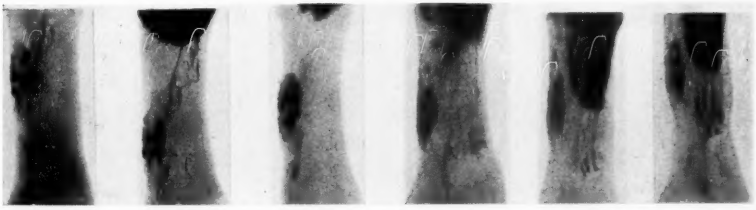

Fig. 4. Fig. 5. Fig. 6. Fig. 7 Fig. 8. Fig. 9.

Fig. 10.

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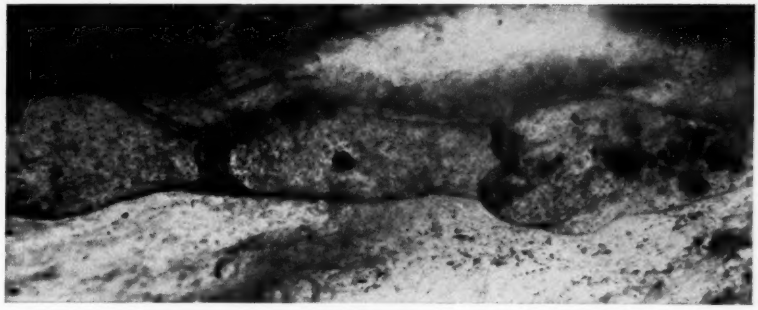

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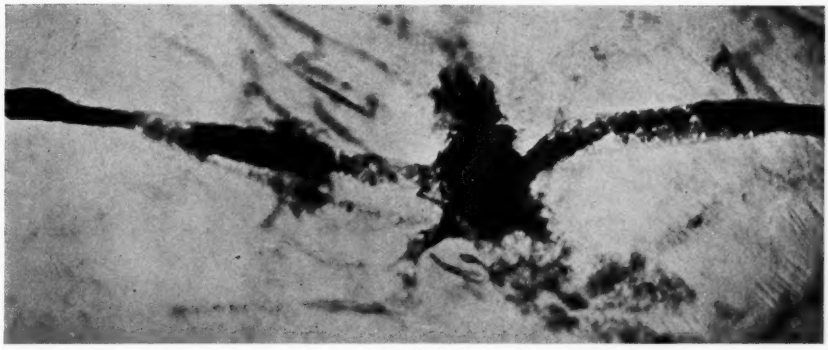

Fig. 2.

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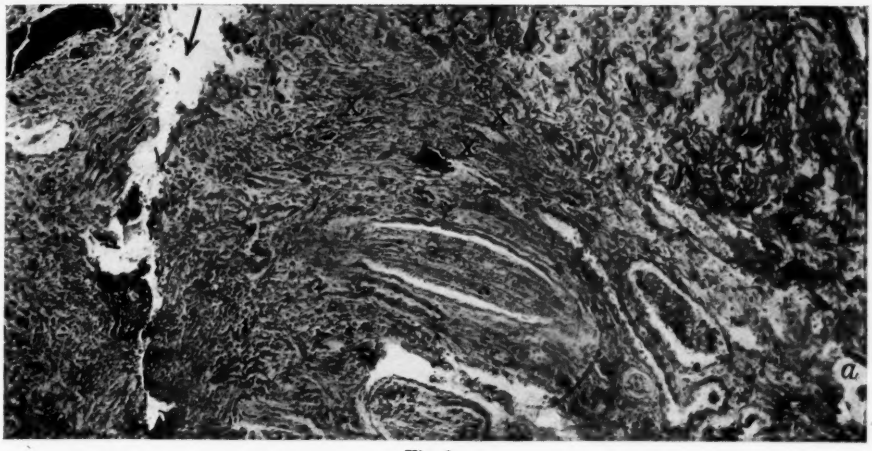

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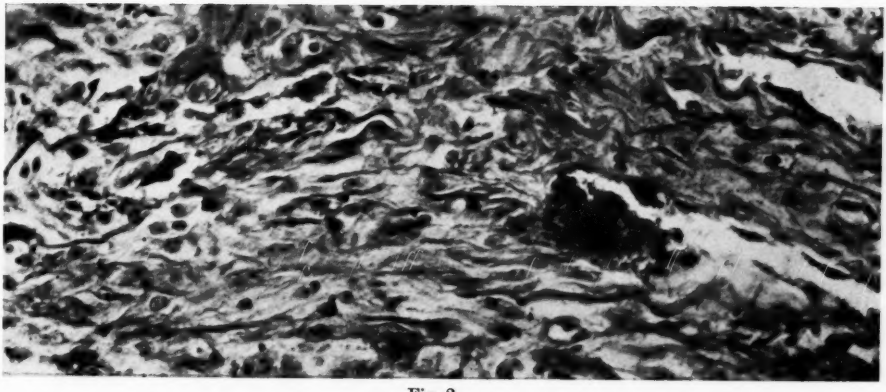

Fig. 2.

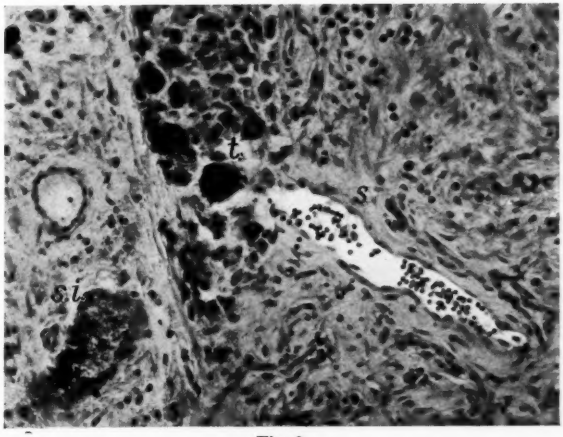

Fig. 3.

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Fig. 3. Radiograph of a rabbit pedicle 2 min. after thorotrast injection of its lymphatics, the base of the pedicle being firmly compressed to obstruct the flow of lymph while massage was employed.

Fig. 4. Radiograph of a rabbit pedicle (R 4) 2 min. after injection with thorotrast.

Fig. 5. Radiograph of the same pedicle (R 4) after thorotrast injection followed by massage of the injected area.

Fig. 6. Radiograph of the same pedicle (R 4) 20 min. after thorotrast injection. Lymphatics now practically completely empty.

Fig. 7. Radiograph of the same pedicle (R 4) injected with thorotrast 8 days after the operation of severing and reunion.

Fig. 8. Radiograph of the same pedicle (R 4) 17 days after the operation, but without further injection.

Fig. 9. Radiograph of the same pedicle (R 4) reinjected 3 weeks after the severing operation.

Fig. 10. Radiograph of the same pedicle (R 4) at the time of killing and finally reinjecting the lymphatics 27 days after the severing operation.

Plate II

Fig. 1. Photomicrograph of an ink-injected normal lymphatic trunk in the rabbit's ear showing the type of vessel operated on. The ear was cleared by the Spalteholz method. The tissue was unstained. This segment of the vessel contains only a few particles of ink. ×25.

Fig. 2. Photomicrograph of an ink-injected lymphatic trunk in the rabbit's ear 6 days after severing. Reunion with an excess of sprouts has occurred. ×20.

Fig. 3. Photomicrograph of lymphatic trunks in a rabbit's ear showing failure of union of a vessel severed 14 days previously. The cut ends were left about 1·0 mm. apart, the vessels were uncovered during the operation by lifting up a flap of skin for inspection. Reunion has failed to occur. Histological examination showed that sprouts (not filled with ink) from the cut ends were present. (*X*) marks the cut ends. ×15.

Plate III

Fig. 1. Photomicrograph of a longitudinal section through area of isolated lymphatic trunks in a rabbit's ear; animal killed 6 days after operation. No injections until the time of death. Section shows the upper (distal) edge of the rectangular line of incision indicated by an arrow, and a small part of the normal distal tissue. Sprouts, *X*, are seen from the distal ends of the isolated trunk (*a*) growing distally. ×85.

Fig. 2. High power view of part of Pl. III, fig. 1. This shows several of the sprouts growing distally. Their endothelium and the contained ink can be recognized. ×350.

Fig. 3. Photomicrograph showing junction of a lymphatic capillary sprout (*s*) with the reversed transplanted trunk (*t*) shown also in Text-fig. 11. The sprout at this point contains chiefly lymphocytes. Ink was seen in it in other sections. The trunk contains phagocytosed ink. An ink-containing sprout is seen at *s.i.* ×80.

THE SPINAL ACCESSORY NERVE IN THE SHEEP

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Department of Anatomy, University of Cambridge

INTRODUCTION

THE spinal accessory nerve has given rise to much controversy, first, because of its strange course, and secondly, because of the doubt as to whether the nerve is purely motor or contains sensory fibres as well.

As recently as 1935 Edgeworth stated that the spinal accessory nerve is purely motor in function and "usually does not contain any ganglion cells". However, Weigner (1901) and Fahmy (1927) found cells of a sensory type on the intracranial and extracranial portions of the spinal accessory nerve in man, and the latter has noted that the cells are more numerous in children than in adults. Windle (1931*b*), by studies of chromatolysis, has found many sensory cells on the intracranial and extracranial portions of the spinal accessory of the cat and monkey, and also spinal accessory cells in the first cervical dorsal root ganglion of the cat. In the first cervical segment of the cat he has traced sensory fibres, via the dorsal roots and the spinal accessory roots, into the caudal part of the solitary tract (1931*a*).

Weigner (1901), Streeter (1905), Pearson (1938) and others have shown nervous connexions between the dorsal roots and ganglia of the first two cervical spinal nerves and the related parts of the intradural spinal accessory nerve in man. Similar connexions for the cat have been suggested by Windle (1931*b*).

From studies on human embryos Streeter (1905) has shown that the vagus and spinal accessory nerves are derived from the same complex, and that their sensory cells take origin from one mass of ganglion crest material. He has also shown that many of the sensory fibres of the spinal accessory nerve are incorporated in the dorsal root ganglia of the cervical nerves. Straus & Howell (1936) put forward this and other evidence to suggest that "there is a marked tendency for sensory cells of XI to shift over to the spinal nerves", and they suggest that the motor fibres of the spinal accessory nerve also tend to follow the spinal course, but to a lesser degree in the animals studied, using this to explain the apparently anomalous innervation of the trapezius group of muscles. From this it would seem almost certain that the spinal accessory nerve contains a sensory component, but the exact nature of these sensory fibres is not so clear, though they are probably proprioceptive fibres from the trapezius group of muscles and do not appear to carry any sensation of pain (Chauveau, 1891; Windle & De Lozier, 1932).

There are two main points of view concerning the phylogenetic development of the spinal accessory nerve:

(1) That the motor nucleus of the spinal accessory nerve is derived from the somatic motor column of cells in the upper cervical spinal cord, and that

its cells and their processes have moved away from the anterior horn and root respectively (Addens, 1933) to supply a functionally separate somatic muscle entity.

(2) That the cells of the motor nucleus of the spinal accessory nerve are derived from the primitive dorsal nucleus of the vagus by a caudo-ventral migration, similar to the ventral migration forming the nucleus ambiguus (Kappers, 1920; Straus & Howell, 1936); and that the muscles which it supplies are visceral or special branchial in nature (Edgeworth, 1935; Straus & Howell, 1936).

There is much evidence, including that of Vermeulen on the llama and camel, to suggest that the latter view is correct and that the former is open to considerable criticism. However, there are still many points which need clarification.

MATERIAL AND METHODS

Macroscopic dissections of the spinal accessory nerve were made in three adult and two foetal sheep. The intradural parts of two nerves from an adult sheep and one from a foetal sheep 111 days old, and the extradural part of one nerve from this foetal sheep were fixed in 10 % formalin, imbedded in paraffin wax, serially sectioned at 10 μ, and stained with toluidin blue.

The medulla and upper cervical spinal cord of five foetuses of various ages, 78–124 days inclusive, were fixed by injection with 10 % formalin, mordanted for at least 1 month in Weigert's primary mordant, serially sectioned in celloidin at 50 μ and stained by the Weigert-Pal technique. To remove the stain from the celloidin prior to differentiation $\frac{1}{2}$ % caustic soda was used. This was found to act rapidly on thick sections and to give as satisfactory a picture as lithium carbonate.

Measurements of cells and fibres were made with an $\times 8$ micrometer eyepiece and a $\frac{1}{12}$ in. oil immersion objective. In the case of nerve cells only those showing a large area of nucleus containing a central nucleolus were measured, the greatest diameter being measured first, then the diameter at right angles to this.

Cell counts in the spinal accessory ganglia were made by counting the cells showing a definite nucleus in every third section, i.e. every 30 μ. As this is slightly greater than the diameter of the nucleus of the medium-sized cells, an approximation to the total number of cells was obtained, though it is possible that some large cells may have been counted twice and many small cells missed.

THE INTRADURAL PART OF THE SPINAL ACCESSORY NERVE IN THE SHEEP

The nerve extends between the dorsal and ventral roots from a position just ventral to the entry of the dorsal roots of the sixth cervical nerve to the dorsal aspect of the ligamentum denticulatum at the first cervical segment. The general course of the nerve is therefore cranial and ventral.

In its cephalic part the cranial (bulbar) root arises, by some ten roots, just ventro-lateral to the nucleus cuneatus, and caudal to this the spinal roots arise in an irregular manner, being most numerous opposite the roots of the cervical spinal nerves and larger in the upper than in the lower cervical region.

The spinal roots are very numerous and are of two distinct types (Pl. I, fig. 1):

(1) Passing from the cord in a position just ventral to the dorsal spinal roots and entering the dorsal aspect of the trunk of the spinal accessory nerve.

(2) Passing from the cord closer to the ligamentum denticulatum, and entering the ventral aspect of the nerve trunk.

The first dorsal type is found throughout the length of the spinal accessory nerve, while the second ventral one only extends as far caudally as the third or fourth cervical segment. The two types of root do not join before entering the nerve, but many more specimens would have to be studied before any general conclusion could be obtained.

The arrangement of the roots is interesting. Several of the dorsal roots are frequently found to join into a common trunk before entering the spinal accessory nerve. The ventral roots, however, are all single in the sheep studied.

Macroscopically it is not easy to identify any nervous connexions between the intradural part of the spinal accessory or its rootlets and the cervical spinal roots or ganglia, because the trunk of the nerve is further removed from the first cervical ganglion than from any other, and does not touch it as in the human (Pearson, 1938).

In one of the sheep studied a small filament could be seen passing from the spinal accessory to one of the dorsal roots of the first cervical nerve; in the serial Weigert-Pal sections of the 111-day-old sheep foetus, a small filament could be traced from the spinal accessory to the dorsal root of the first cervical nerve. On account of its faint staining and minute calibre, it is impossible to figure it here; its nervous nature is questionable and it is not visible in any of the other segments.

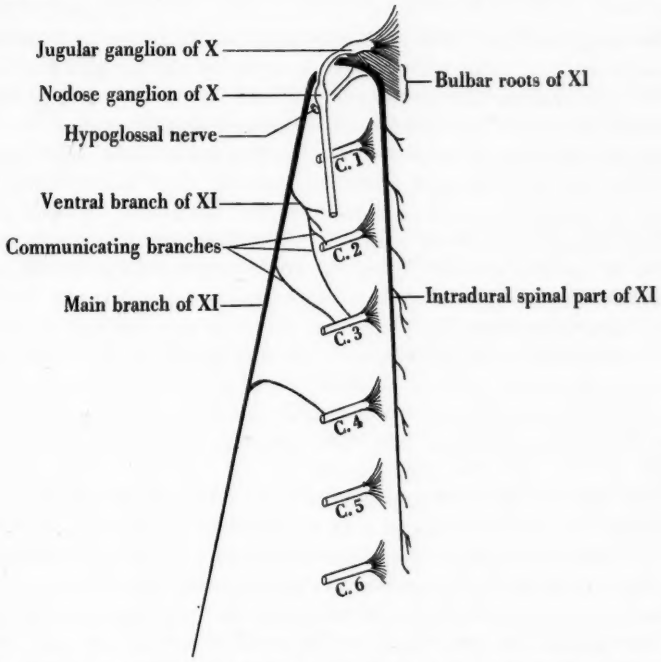
It would appear then that connexions between the intradural part of the spinal accessory and the cervical spinal roots are not frequent or of large calibre.

THE EXTRADURAL PART OF THE SPINAL ACCESSORY NERVE IN THE SHEEP

At its cranial end the accessory nerve runs ventro-laterally, coming to lie just caudal to the vagus close to its jugular ganglion. These two nerves pass out in the same dural sheath, and though interconnecting fibres can be seen, the spinal accessory remains distinct throughout its course in the jugular foramen. Outside the foramen the vagus turns caudally and the spinal accessory, leaving it proximal to the nodose ganglion, runs caudo-laterally and is immediately separated from it by the hypoglossal nerve. The accessory nerve then lies between the submaxillary gland and the rectus capitis lateralis

muscle and caudal to the paramastoid process. Distally it passes on to the ventral surface of the lesser oblique muscle and here divides into two branches:

(1) One, passing ventrally, divides to surround the musculo-tendinous parts of the sterno-maxillarius and the slip from the cephalo-humeral muscle, as they pass around the anterior belly of the omohyoid muscle to join the longus colli; this branch enters these muscles. The most caudal of the two branches receives anastomotic fibres from the second and third cervical nerves (Text-fig. 1).



Text-fig. 1. Diagram to show the relation between the spinal accessory nerve, the vagus, hypoglossal, and spinal nerves, and its connexions with the latter, as seen in ventral view.

(2) The other branch passes caudo-dorsally on the medial surface of the superficial part of the cephalo-humeral muscle and is closely applied to it. Shortly after reaching this muscle the nerve receives an anastomotic filament from the third cervical nerve and, passing dorsally, comes to lie between the superficial and deep parts of the cephalo-humeral muscle, which are, in their caudal part, closely bound together; here a branch from the fourth cervical nerve can be traced into the dorsal branch of the spinal accessory. Traced still farther peripherally the nerve passes from the deep surface of the cephalo-humeral muscle on to the deep surfaces of the cervical and dorsal trapezius

muscles, breaking up into its terminal fibres beneath the latter. Fibres from this main branch can be traced into all the muscles mentioned as close relations of the nerve; and these are supplemented, in the case of the cephalo-humeral muscle, by branches from the second to the fifth cervical nerves. It is not known whether all these branches are functionally related to the muscle or merely pierce it.

THE MOTOR NUCLEUS AND ITS FIBRES IN THE FOETAL AND EMBRYONIC SHEEP

In the foetal sheep of 78 days there is little myelin in the white matter of the spinal cord, and those fibres which do stain by the Weigert-Pal method are finely medullated. The dorsal roots of the spinal nerves contain little myelin, and the ventral roots show only a slightly heavier stain.

From the dorso-lateral aspect of the ventral horn, whose cells show the myeloid reaction of Lénhossek (1889), a group of more heavily medullated fibres can be traced in a cephalo-dorsal direction towards the junction of the ventral and dorsal horns, to be joined by a few fibres from the postero-central region of the ventral horn (Pl. I, fig. 2). At this point, having formed a more compact bundle, they pass laterally to pierce the lateral columns as one or two fascicles. Emerging from the spinal cord they pass in a cephalic direction to enter the intradural trunk of the spinal accessory nerve. In the upper cervical region these fibres lie more in a transverse plane than in the mid-cervical region, so that frequently the whole length of one rootlet is seen in a single section (Pl. I, figs. 1–3) and here there are two distinct pathways to the exterior:

(1) Passing out by running around the ventral aspect of the dorsal horn, and piercing only the most dorsal part of the lateral columns (Pl. I, fig. 3).

(2) Piercing the white matter of the lateral columns throughout its course and passing out close to the ligamentum denticulatum (Pl. I, fig. 1).

These two types of rootlet appear almost in the same plane, are frequently found throughout the upper part of the cervical spinal cord, and are not segmental in their arrangement. In the mid-cervical region usually only one type of rootlet is visible, in a position rather between that taken by the two rootlets in the upper cervical cord. This rootlet is much smaller in the sheep foetus of 78 days.

The fibres of these roots retain this arrangement in the older sheep foetuses, and the study of these only shows an increase in the degree of medullation and the number of fibres medullated. In the 124-day-old sheep foetus, these roots are no longer visible as separate entities more heavily medullated than the surrounding white matter, as by this time the latter has reached an almost adult degree of medullation.

The fibres described above are almost certainly motor in nature, and are the earliest medullated fibres in the spinal accessory nerve of the sheep, and

the most heavily medullated fibres in the cervical spinal cord of the 78-day-old sheep foetus.

As has been seen these fibres arise from a nucleus in the dorso-lateral part of the ventral horn. This is a small, compact nucleus, which in the mid-cervical region lies at the lateral edge of the ventral horn, in a position slightly ventral to that occupied by the intermedio-lateral column of cells in the thoracic region (Text-fig. 2 *b*, *c*, *d*), and sends its fibres dorsally close to the medial edge of the lateral columns. In the upper cervical region the nucleus shifts medially and comes to lie in a central position dorsal to the cells of the ventral horn. The fibres from the nucleus in this region run dorso-medially for some distance before curving laterally to enter the white matter of the lateral columns on their way to the spinal accessory.

Traced farther in a cephalic direction the nucleus is seen to lie in the same column of cells as the nucleus ambiguus, though there does not appear to be any continuity between the two nuclei. In section the motor nucleus shows an average of 5–10 cells of the same size and shape as the other cells of the ventral horn.

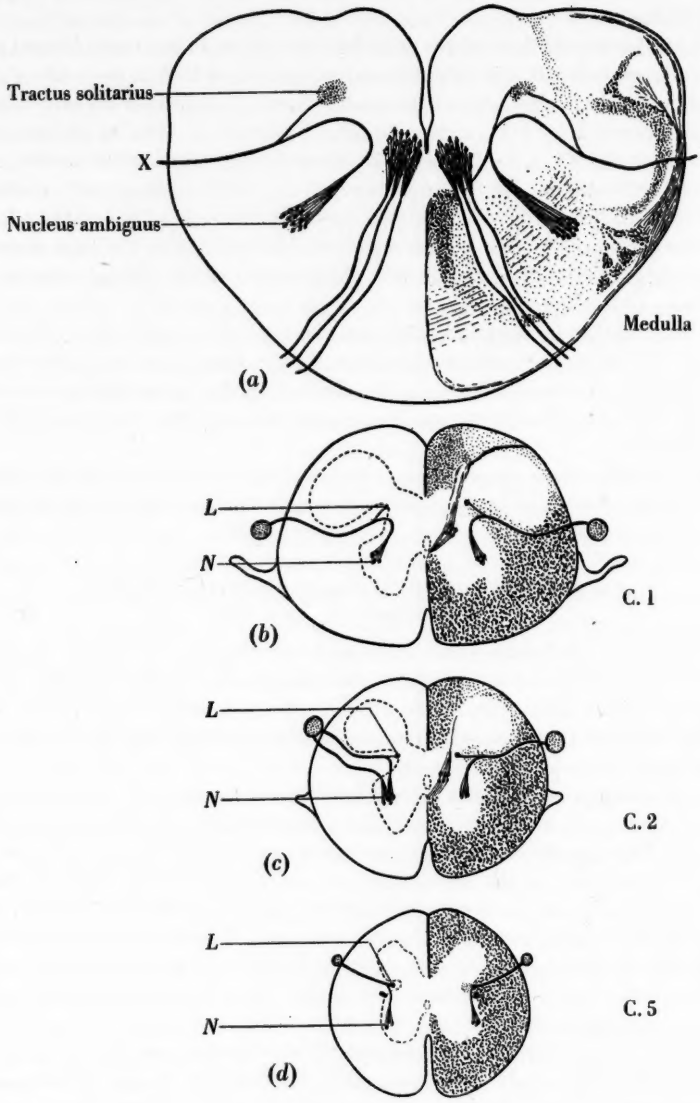
In pyridine-silver preparations of sheep embryos of 25 and 36 days a similar arrangement of the motor nucleus and fibres of the spinal accessory nerve is to be seen.

THE SENSORY PORTION OF THE SPINAL ACCESSORY NERVE

Throughout its course both intra- and extradurally, the spinal accessory nerve of the sheep shows no sign of macroscopically visible swellings of the ganglionic type. However, in the absence of degeneration experiments, it is justifiable to speak of a sensory component, because cells of a ganglionic type are present on the intradural part.

Serial sections through the extracranial part of the nerve in the 111-day-old sheep foetus show no signs of ganglion cells when stained with toluidin blue. Though this is insufficient evidence, it would appear that large ganglia are not invariably present on the extradural part of the nerve, but a few scattered cells may be present in a certain number of cases as described by Fahmy. It is of interest to point out in this connexion that Fahmy shows considerable variability in the number of cells on the spinal accessory, there being only a small number on the extracranial part of one out of three nerves from human specimens of different ages.

Serial sections of the intradural part of three nerves, one from a foetal and the other two from adult sheep, are analysed in Table I. Counts of the number of cells on the nerve and its rootlets have been made, and the distance from the point where the nerve entered the jugular foramen to the group of cells is given, together with an approximate measure of the sizes of the various cells. From these figures it will be seen that the number of cells on the intradural part of the spinal accessory nerve of the sheep is not constant, and though



Text-fig. 2. Diagram, based on the Weigert-Pal stained sections of the spinal cord of a 111-day-old sheep foetus, to show the position and connexions of the motor nucleus, longitudinal tract, and trunk of the spinal accessory nerve throughout the upper cervical region. (*a*) shows, for comparison, the medullated connexion of the vagus at this stage. *L*, longitudinal tract of the spinal accessory nerve; *N*, nucleus of the spinal accessory nerve.

Table I

Nerve	Type of cell group	No.	Distance from jugular foramen, mm.	Cell no. approx.	Cell size μ Max.	Cell size μ Min.
Adult sheep, total length 18 cm.	Ganglia	1	20	320	66 × 66	15 × 12
	Diffuse cell groups	3	(1) 25	25	70 × 42	21 × 15
			(2) 30	1		
			(3) 160	1		
Adult sheep, total length 18 cm.	Ganglia	2	(1) 27	565	80 × 54	24 × 18
			(2) 30	20	80 × 45	30 × 18
	Diffuse cell groups	4	(1) 20	3	60 × 24	45 × 24
			(2) 30	78	80 × 45	30 × 18
			(3) 42	3	51 × 42	33 × 27
			(4) 175	1	60 × 15	
111-day-old foetal sheep, total length 8 cm.	Ganglia	1	5	315	50 × 40	15 × 12
	Diffuse cell groups	3	(1) 5	78	36 × 24	
			(2) 15	16	45 × 30	24 × 18
			(3) 60	10	35 × 18	

many more nerves must be sectioned before a reliable result can be obtained, it appeared that the majority of the cells are confined to the cephalic end of the spinal part of the nerve.

It will be seen that there is in all three nerves studied a small elongated ganglion (Pl. I, fig. 4) lying on one of the cephalic spinal rootlets at the base of which are found a few scattered cells in the trunk of the nerve. Caudal to this the number of cells is very small, suggesting that the sensory fibres in the spinal accessory nerve may be situated in the more cranial rootlets.

The cells mentioned above are of the typical dorsal root ganglion type, and show a considerable range in size and shape. Those in the ganglia are approximately circular in section, and here an almost equal number of small and large cells exist together, while in the diffuse cell groups a more medium size of cell is found.

It is of interest to note that there is no striking difference in the number of cells found in the foetal and adult nerves studied.

In the 84-day-old sheep foetus, in addition to the motor rootlets of the spinal accessory nerve, there is a longitudinal tract composed of a few heavily medullated fibres, lying at the medial edge of the white matter where the motor fibres enter the lateral columns (Pl. I, fig. 5). This bundle is quite irregular in its arrangement, being at times divided up into several fascicles, and varying in the number of fibres which it contains. It receives fibres from the rootlets of the spinal accessory, which turn caudally to run in the tract for a short distance.

In the older sheep foetuses it is possible to note an increase in the number and diameter of the fibres forming the longitudinal tract (Table II).

The tract itself when traced in a cephalic direction in the older sheep

Table II

Age of sheep foetus in days	No. of medullated fibres in the longitudinal tract in the 5th cervical segment	Average diameter of the fibres μ
84	20	2·0
98	40	3·6
111	200	5·0

foetuses is found to thin out and pass dorso-medially in the first cervical segment so as to come to lie in a direct caudal line with the solitary tract. In the Weigert-Pal preparations it has not been possible to identify any continuity between the two tracts before the 124-day-old stage when a small bundle of heavily medullated fibres can be seen joining them. A few connexions can be seen between the longitudinal tract and Stilling's nucleus, but apart from this no other medullated connexions have been visible in the foetuses studied, though Barron & Thomson (1937) report connexions between the dorsal spinal roots and this tract in the pyridine-silver preparations of sheep embryos. It is possible that these fibres medullate late in development.

It has been impossible on the basis of histological study alone to determine the final destination of the fibres in the longitudinal tract, though the irregular nature of the tract, especially in the upper cervical region, suggests only a short caudal course of one or two segments for these fibres.

This longitudinal tract is almost certainly the "caudal continuation of the solitary tract" reported for sheep embryos by Barron & Thomson and for cat embryos by Windle. Both these authors considered that there were sensory fibres of the spinal accessory nerve entering this part of the solitary tract. It has also been noted by Obersteiner (1890) and Cajal (1909), who thought it corresponded to Krause's respiratory bundle (solitary tract), and consisted of motor fibres running in a cephalic direction before passing out to join the spinal accessory nerve. In the sheep this tract does not appear to be formed by motor fibres of the spinal accessory nerve, which do not form any longitudinal tract in the 78-day-old sheep foetus, although at this stage they are heavily medullated.

A series of diagrams based on the anatomy of the 111-day-old sheep foetus (Text-fig. 2) show the connexions of the longitudinal tract and motor nucleus of the spinal accessory nerve. The medullated central connexions of the vagus are shown (Text-fig. 2*a*) in order to indicate the similarity between the sensory fibres of the vagus entering the solitary tract, and the fibres of the spinal accessory rootlets entering the longitudinal tract in the cervical segments.

DISCUSSION

The precocious fibrillar development of the spinal accessory is well known (Windle, 1931*b*; Barron & Thomson, 1937), and running parallel with this is the early appearance of myelin in the rootlets and trunk of the spinal accessory nerve of the sheep.

The first appearance of myelin in the spinal accessory nerve of the sheep is seen in those fibres arising from a discrete nucleus in the postero-lateral aspect of the ventral horn in the 78-day-old sheep foetus. These fibres are almost certainly motor in nature and are at this time the only medullated connexions of the spinal accessory nerve with the spinal cord. The anatomical position of the nucleus in the foetal sheep does not differ greatly from its counterpart in other mammals and, since it is not closely related either to the dorsal nucleus of the vagus or to the hypoglossal nucleus, does not give support to either of the theories, put forward in the first instance by Kappers (1920) and Addens (1933) respectively, suggesting its origin from one of these nuclei. Its close relation, if not continuity, with the nucleus ambiguus, and its position at the postero-lateral aspect of the ventral horn, but slightly ventral to the position taken up by the intermedio-lateral cell column in the thoracic spinal cord, suggests that this nucleus has a closer relation to the visceral than to the somatic column. It should be noted that in the sheep embryo the motor nucleus of the spinal accessory nerve is an integral part of the developing ventral horn even in the youngest sheep studied. But it is at no level situated in a ventro-medial position in the ventral horn, as described by Bruce (1901) for the upper cervical segments in man.

In the older sheep foetuses new connexions appear which develop their myelin more slowly. They consist of fibres running from the spinal accessory to a longitudinal tract situated at the lateral edge of the grey matter at the junction of the ventral and dorsal horns. These fibres enter this tract and run within it for a few segments in a caudal direction. Without recourse to degeneration experiments it is not possible to say what their final distribution is, but it is probable that they represent the sensory component of the spinal accessory nerve which has been traced into the caudal continuation of the solitary tract in silver preparations of sheep embryos (Barron & Thomson, 1937) and cat embryos (Windle, 1931 *a*). These fibres probably end in the nucleus of this longitudinal caudal portion of the tractus solitarius (Straus & Howell, 1936). However, no definite nucleus related to this tract has been noted other than the nucleus of Stilling with which the tract has a few connexions. It is to be noted that the sensory component medullates much later than the motor fibres of the spinal accessory nerve and in this respect resembles the spinal roots in the sheep (and in the cat and human (Langworthy, 1928, 1933)) in which the motor roots are medullated before the sensory roots. In the sheep, there is no evidence of reduction in the size of the spinal accessory nerve, nor of the transference of motor fibres from the spinal accessory to the spinal nerves, as suggested by Straus & Howell and described in a varying degree in certain ungulates (Lesbre, 1903; Todd & Todd, 1912; Vermeulen, 1914; Zuckerman & Kiss, 1932; Kiss & Zuckerman, 1934).

It is thought that the sensory cells which lie on the trunk of the spinal accessory nerve (see also Kazzander, 1891; Weigner, 1901; Streeter, 1905; Fahmy, 1927; Windle, 1931 *b*; Pearson, 1938) send their fibres into the longi-

tudinal tract, as has been previously described (Windle, 1931; Barron & Thomson, 1937). This tract, which has long been known to be a caudal continuation of the solitary tract (Obersteiner, Cajal, etc.), was regarded as a motor component of the spinal accessory nerve. In the sheep both the delayed myelination of these fibres, as compared with the motor fibres of the spinal accessory nerve, and the course of the tract including its close relation and subsequent myelinated connexion with the solitary tract, suggest that this tract differs from the motor part of the spinal accessory nerve and is probably sensory in nature. It is not intended to suggest that the solitary tract is functionally related to the longitudinal tract of the spinal accessory nerve, for the great difference in the time of medullation of these two parts suggests a functional if not an anatomical separation. The solitary tract is but slightly medullated in the 111-day-old sheep foetus, by which stage the longitudinal tract of the spinal accessory nerve is heavily medullated.

The lack of medullated connexions between the fibres of the longitudinal tract of the spinal accessory nerve and the cervical nerves which anastomose peripherally with this nerve is strange, since it has been shown that these anastomotic branches carry the majority of the proprioceptive fibres from the trapezius and sternomastoid muscles in other animals (Corbin & Harrison, 1939; Yee *et al.* 1939).

It is of interest to note the close resemblance between the results obtained above in the sheep and those obtained by Pearson working on human material.

SUMMARY

1. Descriptions of the macroscopic arrangement of the spinal accessory nerve in the sheep are given, with special reference to its relationship to the cervical spinal nerve roots.

2. Two distinct stages of medullation exist in the spinal accessory nerve.

(*a*) Fibres from the motor nucleus of this nerve are already well medullated in a 78-day-old sheep foetus.

(*b*) A second set of fibres connecting the spinal accessory nerve with a longitudinal tract, visible as a caudal continuation of the solitary tract in premyelin stages, begins to medullate in an 84-day-old sheep foetus, and reaches adult medullation by the 124th day of foetal life, by which time it shows a medullated continuity with the solitary tract not previously visible.

3. Ganglionic cells of a sensory type have been demonstrated on the intradural part of the spinal accessory nerve and on one or two of its more cephalic rootlets.

4. It is suggested that the spinal accessory nerve of the sheep is a mixed nerve whose medullation is precocious. It appears to be related to the visceral rather than to the somatic cell columns, as suggested by its connexions with the solitary tract and the close relation between its motor nucleus and the nucleus ambiguus.

Fig. 1.

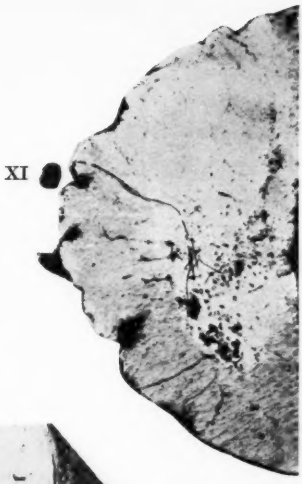


Fig. 2.

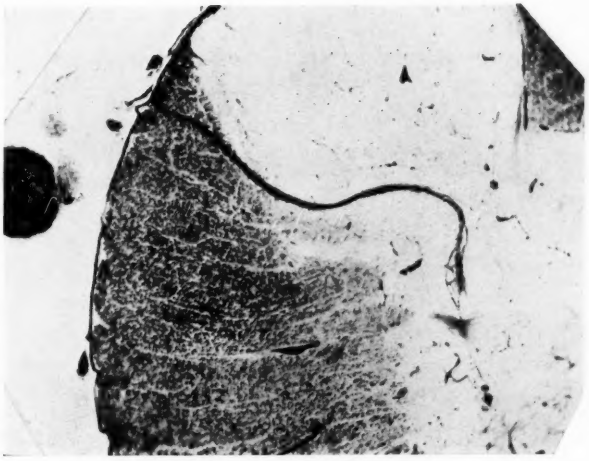


Fig. 3.

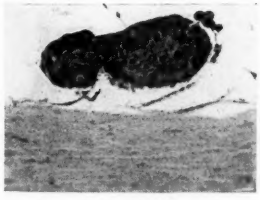


Fig. 4.

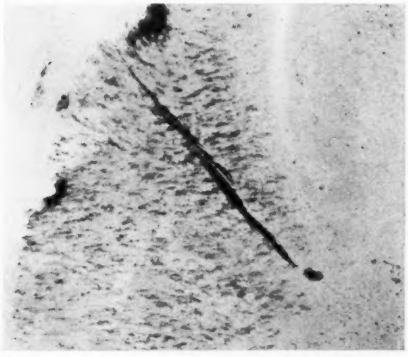


Fig. 5.

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I am indebted to Sir Joseph Barcroft and his co-workers in the Department of Physiology for supplying the sheep material for this study and to Prof. Harris in whose laboratory the work was carried out and whose helpful advice and criticism, together with that of Dr Barron (who first suggested this line of research), has contributed greatly to its completion. I am also indebted to Dr Barron for allowing me to study some of the pyridine-silver preparations of sheep embryos reported by Barron & Thomson (1937).

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EXPLANATION OF PLATE I

Fig. 1. Transverse section through the spinal cord of a 111-day-old sheep foetus in the caudal part of the first cervical segment. Stain, Weigert-Pal. ×14.

Fig. 2. Transverse section through the spinal cord of a 78-day-old sheep foetus in the second cervical segment. Stain, Weigert-Pal. ×50.

Fig. 3. Transverse section through the spinal cord of a 111-day-old sheep foetus in the lateral column region of the first cervical segment. Stain, Weigert-Pal. ×45.

Fig. 4. Longitudinal section through the intradural part of the spinal accessory nerve and ganglion of an adult sheep. Stain, toluidin blue. ×45.

Fig. 5. Transverse section through the spinal cord of an 84-day-old sheep foetus in the lateral column region of the third cervical segment. Stain, Weigert-Pal. ×100.

THE INTRAMURAL NERVOUS SYSTEM OF THE SMALL INTESTINE WITH SPECIAL REFERENCE TO THE INNERVATION OF THE INNER SUBDIVISION OF ITS CIRCULAR MUSCLE[1]

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In a previous communication (Li, 1936, 1937) it has been pointed out that the circular muscle of the small intestine of various vertebrates is subdivided into two layers, a thin inner layer and a thick outer layer. The inner layer is composed of one to several layers of muscle cells which are much smaller in size, sometimes branching and anastomosing with other muscle cells. This layer is richer in nuclei than the outer layer. Since it stands out as a separate entity and consists of a rather primitive type of smooth muscle cell, with occasional ganglion cells, it was suggested that it might be a "neuromuscular" structure and be responsible for irritability or conduction in the small gut. The present study deals with its nerve supply, a point of some importance in developing the neuromuscular idea.

PRESENT STATUS OF OUR KNOWLEDGE

Since very little is known about this subdivision of the circular muscle, nothing is to be found in the literature about its nerve supply. However, a general survey of our present knowledge of intestinal innervation will not be out of place, especially with a view to seeing how the special innervation of this particular layer fits into the general scheme.

The voluminous literature on this subject has been completely reviewed by Hill (1927), Schabadasch (1930) and Patzelt (1936). It is generally agreed that the small intestine, like other parts of the digestive tube, is supplied with nerves of extrinsic and intrinsic origin. Both vagus and sympathetic nerves enter the intestinal wall from without, but their mode of termination and their relationship to the intramural nervous system are not yet clear. According to Stoehr (1932) the intramural nerves of the intestine may be classified into four systems which are, however, very intimately connected: (1) plexus subserosus, which has been very well demonstrated by Schabadasch (1930); (2) plexus myentericus (Auerbach's plexus) lying between the circular and longitudinal muscle layers; (3) plexus muscularis profundus situated within the circular

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muscle (Drasch, 1881; Cajal, 1893); (4) plexus submucosus (Meissner's plexus) situated in the submucosa and extending into the mucosa. There are free anastomosing fibre strands between the myenteric and submucous plexuses.

The myenteric plexus consists essentially of three plexiform mesh works (Schabadasch, 1930; Stoehr, 1930; Greving, 1931), viz. a primary, a secondary and a tertiary plexus. The primary plexus is a relatively coarse structure. Its meshes vary within wide limits in size and form, but in general it exhibits a longitudinal arrangement. The secondary plexus is intimately connected with the primary but is made up of more slender bundles of nerve fibres. The primary and secondary plexuses lie mainly within the connective tissue between the circular and longitudinal muscle layers. The tertiary plexus is a very delicate meshwork of fibres directly connected with the secondary plexus, lying inside the circular muscle and running in the same direction. Its ramifications form a fine meshwork known as plexus muscularis profundus. According to van Esveld (1928*a*) this plexus is again divided into still finer meshes, the "plexus terminal ou interstitiel".

Meissner's plexus consists of several superimposed meshworks of nerve fibres and ganglia and has been described in the literature (Stoehr, 1930; Schabadasch, 1930) as composed of two plexuses, the plexus entericus internus (Henle's plexus) and plexus submucosus (i.e. the plexus submucosus in its narrow sense or Meissner's plexus proper). The plexus entericus internus is situated at the extreme periphery, that is close to the circular muscle. It differs considerably from the structure of Auerbach's plexus in the delicacy of its bundles and ganglia, as well as the irregularity in size and arrangements of its meshes. The plexus submucosus (in its narrow sense) is situated near the muscularis mucosae and is much finer in its meshes and elements than those of Henle's plexus.

It has long been known that in addition to the plexuses of Auerbach and Meissner and their nerve cells, there exists in the digestive tract another plexus consisting of anastomosing stellate and spindle-shaped cells with varicose processes. This "cell net" is often named the interstitial cell net of Cajal who first described it (Cajal, 1889, 1893). Uncertainty exists as to the nature of these interstitial cells and there are two contradictory views:

(1) *Nervous tissue view.* Cajal (1889) demonstrated the presence of a neurofibrillar network in these cells, varicosities on their processes and their deeply staining character with methylene blue. All these facts led Cajal and his pupil La Villa (1897) to consider them as nervous in character. Both of these authors failed, however, to demonstrate any connexion with extrinsic and intrinsic nerve fibres. P. Schulz (1895) and E. Mueller (1920) regard them as sympathetic nerve cells. Bethe (1903) and Prentiss (1904) consider them as a peripheral nerve cell net. Tiegs (1925) describes a nerve cell net of the gut wall (apparently identical with these cells) and he believes that it is the seat of the so-called myogenic contractions of the gut wall. Woollard (1926), in his study on the innervation of blood vessels, has observed a similar type of cell

net both in large and small vessels. He makes no comment on the nature of this cell net but emphasizes the fact that the large branching cells are closely related to the unmyelinated nerve fibres and that they persist after denervation by removal of the sympathetic trunk. Lawrentjew (1929) claims to have demonstrated nerve fibres in these cells and, upholding Held's theory, regards them as lemmoblasts. He considers the interstitial cell net as a syncytial structure, and holds that it functions as the end-part of a protoplasmic conducting tissue of the autonomic nervous system ("als Endglieder einer protoplasmatischen Leitungsbahn des autonomen Nervensystems"). This lemmoblastic concept has been well received by many authors (van Esveld, 1928*a*; Kolossow & Sabussow, 1928; Schabadasch, 1930; Stoehr, 1930) but not without modifications. Stoehr (1932) and his pupils (Reiser, 1933; Riegele, 1932) think that these interstitial cells are identical with the Schwann's cells and interpret them as the conducting tissue (Leitgewebe) or the conducting plasmodium (Leitplasmodium) of the peripheral nerve fibres. They consider this conducting tissue together with the nerve fibres embedded in its protoplasm as a nervous terminal plasmodium. Whether the cells can be considered as neurones is regarded as a question of minor importance. Schabadasch entertains the opinion that these cells may mean something more than a mere covering cell (lemmoblast) and be the generator of the neurofibrillae included in them. Boeke (1935*a*, *b*), while admitting the lemmoblastic theory, says that they may be comparable to the primitive ganglion cells of invertebrates as originally suggested by Cajal. Leeuwe (1937), working in Boeke's laboratory, has recently shown the ganglion cell nature of the interstitial cells with certainty. With methylene-blue staining he could trace syncytial connexions of these cells not only with the ordinary ganglion cells but also with all transitional forms between interstitial cells and ganglion cells. Within the cell body there is a neurofibrillar network which in his opinion is formed by the interstitial cell itself. Thus, the interstitial cells are true ganglion cells, and are not like lemmoblasts merely carrying fibres. Interstitial cells are usually well stained by intravital methylene blue, while Schwann's cells are not stained. Furthermore, they contain Nissl's granules which are stained with methyl green pyronin stain and thionin like ordinary ganglion cells. They show oxidase and peroxidase reactions similar to those in the ordinary ganglion cells. By staining for Reich's π-granules it has been made evident that they are distinct from lemmoblasts (Schwann's cells). In the intestine the varicose processes of the interstitial cells form a periterminal network around the muscle fibres and merge into their protoplasm. In a study of frog larva, he finds that they are genetically related to the sympathetic ganglion cells. Meyling (1938) not only supports Leeuwe's view but goes even one step further in putting forward the suggestion that the interstitial cells may be sensory or motor in character. According to him, sensory cells have thin, broad, lamellar, protoplasmic processes (comparable to the dendrite lamina of Lawrentjew (1929)), while motor cells have much narrower varicose protoplasmic processes.

(2) *Connective tissue view.* Dogiel (1895), being unable to find neurofibrillae in these cells or to trace any connexion between them and the nerve fibres of the enteric plexus, regards them as connective tissue cells. Huber (1897), Ebner (1889–1902), Heidenhain (1911), Kuntz (1922), Tello (1922), Johnson (1925) and Oshima (1929) all fail to find neurofibrillae in these cells, and accordingly support Dogiel's view that they are some peculiar type of connective tissue cell. Thus the arguments for this view are based mainly on negative findings.

It seems that positive facts are accumulating in favour of the view that the interstitial cells are primitive ganglion cells and that they are an important constituent of the enteric nervous system.

MATERIAL AND METHODS

The small intestine of rabbit, guinea-pig, cat and dog was studied by the intravital methylene-blue and the Gros-Bielschowsky stain.

Intravital methylene-blue stain

The animal was killed by a blow on its head, and its blood was washed out with 0·72 % NaCl solution through the thoracic aorta. When the outflow from the inferior vena cava became clear, methylene-blue solution was substituted. The staining solution was made up as follows: methylene-blue 0·25–0·33 g., sodium chloride 7·2 g., distilled water 1000 c.c. The distilled water was first boiled to 100° C. to expel the carbon dioxide and then cooled down to 37° C. Gurr's methylene-blue "medicinal" proved satisfactory. I found that the addition of 0·2–0·3 g. of resorcin to each litre of the dye quickens the process of staining as described by Schabadasch (1935). For the guinea-pig 1000–1500 c.c., for the rabbit and cat 2000–3000 c.c. of the dye were used. After the injection the abdominal cavity was opened and the intestine exposed to the air. Within 10 min. the nerve plexuses were intensely blue, standing out clearly. The intestine was then taken out and fixed in 5–8 % solution of ammonium molybdate.

Spreads were prepared from the well-fixed tissue by separating different layers of its coats. The specimens were mounted in sandarac in eucalyptus oil. I am grateful to Prof. J. P. Hill for this method. The preparation is brought from water to 70 % alcohol, to acetone 3 parts plus eucalyptus oil 1 part, to pure eucalyptus oil and finally mounted in sandarac in eucalyptus oil. Dioxan was also tried—from water to two changes of dioxan, then to oil, and mount in sandarac. These methods prove very satisfactory because the decolorization and shrinkage caused by alcohol and xylol are avoided. For paraffin sections I used dioxan for dehydration and clearing before embedding, as methylene-blue is barely soluble in dioxan.

Gros-Bielschowsky's method

Both Denny-Brown and Richardson's modification were used after fixation in 10% formol plus 2% pyridine, 20% neutral formalin and Lawrentjew's A.-F.-A. mixture. Frozen tangential and transverse sections of cat's and dog's intestine were stained with silver and counterstained with Ehrlich's haematoxylin and eosin. Strips of rabbit's intestine were also stained in the same way. Strips of the longitudinal muscle were easily stained, while the circular muscle and submucosa very seldom gave satisfactory results because of a too dark background. Lawrentjew's fixing mixture followed by silver was specially valuable for the interstitial cells of Cajal.

OBSERVATIONS

On the inner layer of the circular muscle

In flat silver preparations of rabbit's and guinea-pig's small intestine, Meissner's plexus does not appear to be divided into Henle's plexus and Meissner's plexus proper. It is closely applied to the inner layer of the circular muscle, and is composed of interlacing nerve-fibre bundles which are thinner than the fibre strands of Auerbach's plexus. The shape of the meshes may be triangular, quadrangular or polygonal. The ganglia are usually found at the intersections of its anastomosing branches, and are either fusiform or triangular. They contain only a few ganglion cells.

Thick fibre bundles of this plexus send off thinner fibre strands which run mainly in the direction of the circular muscle fibres and anastomose among themselves. The nerve fibres are accompanied by cells and form finely reticulated structures similar to the sympathetic ground plexus of Boeke and the terminal reticulum of Stoehr. Individual nerve fibres have never been noticed leaving a fibre strand to supply the muscle fibres directly. The reticular network is, however, closely associated with the interstitial cell of Cajal. The interstitial cell not infrequently has a process inside the reticulum, taking part in the formation of this fine network. Other processes of the interstitial cells anastomose freely with each other. Some of them seem to end in close relation to the muscle fibres, but the mode of ending cannot be determined.

In methylene-blue flat preparations of the circular muscle, numerous nerve fibres are found within its inner layer in addition to the branches of the overlying Meissner's plexus. Surrounding nearly every muscle fibre there are nerve fibres. Most of them are smooth but slightly wavy, taking a more or less straight course along the muscle fibres. Some of them are definitely varicose. They branch infrequently and anastomose among themselves. These fibres are of local origin; they are the protoplasmic processes of a type of interstitial cell of Cajal. The cells are mostly spindle-shaped and bipolar, less frequently tripolar or multipolar. The cell body is stained less deeply with methylene-blue

than the interstitial cells attached to the longitudinal muscle to be described later. The whole picture resembles what Schabadasch (1930) described as the *plexus muscularis profundus*. Schabadasch places this plexus between the circular and longitudinal muscle layers in the colon, but I believe it to be in the inner layer of the circular muscle of the small intestine. This observation on flat preparations is confirmed in paraffin sections. Both in transverse and longitudinal sections the inner layer of the circular muscle stands out as a blue band because of its richness in nerve fibres and interstitial cells, both of which stain blue.

In silver preparations of cat's intestine, the inner layer of the circular muscle is almost stained black in contrast with the outer layer which remains colourless. There is a fine meshwork which is formed by the anastomosing protoplasmic processes of stellate cells. Fine deeply stained black fibrils (apparently nerve fibres) are sometimes found within the stellate cells. Some of these fibres can be traced back into Meissner's plexus. In tangential sections where this layer is caught in big sheets (Pl. I, fig. 1), one is impressed by the fact that this inner layer contains many more nuclei than the outer layer. The anastomosing cell net is better seen. The stellate cells have a small amount of cytoplasm with oval nuclei devoid of nucleoli. The nuclei are mostly arranged in the direction of muscle fibres, and the anastomosing processes are rather broad and lamellar in appearance. At first glance these cells are not dissimilar to the connective tissue cells found in the boundary zone between the submucosa and the circular muscle by van Esveld (1928*a*, Abb. 18), but C. J. Hill agrees with me that they are interstitial cells. Through the kind introduction of Prof. Hill, I have recently had the opportunity of showing Prof. Boeke my preparations, and he remarked on the similarity of these cells to the interstitial cells described by Meyling (1938) in connexion with the carotid sinus. Their nervous nature can hardly be doubted when one admits that the interstitial cell is a primitive type of ganglion cell (Boeke, 1935 *a*, *b*; Leeuwe, 1937). Fine fibrils are sometimes seen inside this protoplasmic network. They seem to be of local origin and produced by the interstitial cells. Nerve fibres from Meissner's plexus are frequently traced into this layer forming a separate network but closely related to the interstitial cells. The relationship may be best expressed by Prof. Woollard's (1926) description of the nerve supply of blood vessels: "These cells vary in form and seem sometimes to be in apposition with the naked axis cylinder; again they appear to be at the end of a naked cylinder as though this was inserted into the cell; often they appear to be more independent of the axis cylinder. They are characterized by giving off complicated processes which entwine with the axis cylinder." It is a striking fact that definite nerve endings have not been observed. In the dog's intestine a similar black-staining network is present in this layer, but the protoplasmic processes are not so broad. The cells are bipolar, tripolar or, less frequently, multipolar, and are quite typical of interstitial cells.

Outer layer of circular muscle

In methylene-blue preparations of rabbit and guinea-pig intestine (longitudinal and outer circular muscle coats together) which we viewed from the serosa side, strands of nerve bundles of different calibre can be seen forming the primary, secondary and tertiary plexuses described by Schabadasch (1930). Nerve strands of the tertiary plexus run in the outer layer of the circular muscle and mainly in the direction of the muscle fibres. In the interstices of the tertiary plexus there is a loose network of the interstitial cells of Cajal. Protoplasmic processes of the interstitial cells join the finest fibre strands of the tertiary plexus as well as the feltwork of interstitial cells between the circular and longitudinal muscle layers. A few of the varicose processes seem to end in relation to the muscle fibres as end-knobs. Whether these are the actual endings or the torn ends of the processes or partially stained processes cannot be decided.

Longitudinal muscle layer

In stripped preparations Auerbach's plexus is usually attached to the inner surface of the longitudinal muscle coat. There is also a dense feltwork of interstitial cells of Cajal. These form a sheath to the nerve strands of Auerbach's plexus; their protoplasmic processes contribute to the bulk of the nerve strands. Between the meshes of Auerbach's plexus the interstitial cells form a continuous network. Fibre strands from the nerve trunks are frequently seen connected with the meshwork of interstitial cells; in other words, the fibres of the interstitial cells enter into the composition of the fine nerve-fibre strands. The cell bodies are as a rule arranged in the direction of the longitudinal muscle fibres. The whole picture of this feltwork (Pl. I, fig. 2) reminds one of the subserous plexus of Schabadasch (1930) in spite of the topographical discrepancy. His plexus is located in the subserosa (i.e. on the outer surface of the longitudinal muscle), and is composed of anastomosing interstitial cells. He described nerve endings passing from this plexus to the longitudinal muscle. I have found no plexus of a similar type in the subserosa.

Morphology of the interstitial cells and their relationship to the fibre strands and ganglion cells of Auerbach's plexus and the muscle cells

The interstitial cells (Pl. I, fig. 2, and Pl. II, figs. 3, 4) are best seen in the flat preparation of the longitudinal muscle of specimens stained by the intravital methylene-blue and the silver impregnation methods. Morphologically they conform to the description of Hill (1927), van Esvèld (1928*a*) and Schabadasch (1930). They are stellate or spindle-shaped cells with two to five protoplasmic processes without any differentiation into axon and dendrites. The processes are usually very long and branching and anastomosing among themselves. Sometimes a side branch is seen to end in the muscle as a varicose

swelling, in the manner shown by van Esveld (1928*a*, Abb. 16). Endings are, however, very infrequently seen. These processes give different pictures in methylene-blue and silver preparations. In methylene-blue stained specimens they usually show numerous varicosities and are indistinguishable from the varicose processes of the ganglion cells. In silver specimens there is no sign of varicosity and they look like the ordinary nerve fibres in spite of their thick calibre. At the bifurcation there is a triangular knot which gives the typical picture of Remak's knot of nerve fibres. Unless they are traced to the cell body, it is almost impossible to distinguish fibres of interstitial cells and those of the true ganglion cells. I have seen not infrequently in methylene-blue preparations nerve fibres with alternate smooth and varicose segments. The cell body contains comparatively little cytoplasm in which there are granules staining deep blue with methylene-blue. The nuclei are oval in shape, devoid of nucleoli, and deeply stained with silver.

Between the fibre strands and the ganglion cells of Auerbach's plexus and the interstitial cells, a definite relationship exists. The interstitial cells form a dense network around the trunks of nerve strands of Auerbach's plexus. The fibres of the interstitial cells enter the fine fibre strands of the Auerbach's plexus; very frequently one sees that the fine fibre strands break into individual fibres which anastomose with those of the interstitial cells. In Pl. II, fig. 3, are shown the processes of a ganglion cell and interstitial cells forming a loose network where synaptic connexions may occur. Fibres of the interstitial cells form an interlacing network within the fibre strands and around ganglia of the Auerbach's plexus (Pl. II, fig. 4). It is highly probable that synaptic connexions between the two cell systems may occur there.

DISCUSSION

The inner layer of the circular muscle is especially rich in nerve fibres, some of which can be traced to Meissner's plexus. They form a fine meshwork and seem to end here with the intervention of the interstitial cells. The majority of the fibres in this layer are, however, of local origin. They are the protoplasmic processes of interstitial cells of Cajal. They stain with methylene-blue and silver. They are smooth or varicose, or slightly wavy, running a parallel course with the muscle fibres. They differ from elastic and reticular fibres, which are also numerous in this layer, in their staining properties and their arrangement. The elastic fibres in this layer form a dense network which is the continuation of the elastic network in the submucosa. The reticulum runs parallel to the muscle cells but gives off numerous side branches to embrace the muscle cells. The interstitial cells giving rise to fibres appear to me to be more primitive than the interstitial cells in the outer coat which are discussed later. They are usually bipolar, less frequently tripolar and multipolar, and contain fewer granules stainable with methylene-blue. Their processes are rather smooth and less varicose. All of these facts are in favour of the suggestion that they are very primitive.

This plexus of interstitial cells seems to have very little connexion with Auerbach's and Meissner's plexuses but is more closely related to the interstitial cells in the neighbourhood. By means of their protoplasmic processes a connexion is established with interstitial cells in the submucosa and the outer layer of the circular muscle. Furthermore, they communicate with the dense interstitial cell net between the circular and longitudinal muscles at the place where the inner layer of the circular muscle dips down.

Both in the rabbit's and guinea-pig's intestine, where the inner layer of the circular muscle is thin, the interstitial cells and their fibres form a plexus which recalls the picture given by Schabadasch of plexus muscularis profundus in spite of the topographical difference. This author did not state clearly the origin of the fibres of this plexus.

In the cat's and the dog's intestine, where the inner layer of the circular muscle is thick, there is a dense network of interstitial cells, but the picture is somewhat different. In the dog's intestine the interstitial cell net in this layer contains bipolar as well as tripolar and multipolar cells. In the cat's intestine the interstitial cell net in this layer resembles the interstitial cells described by Meyling in the carotid sinus. The anastomosing protoplasmic processes are thin, but broad and lamellar. It is interesting to note that Meyling's sensory interstitial cell net of the carotid sinus is embedded between the elastic fibres. It may be pointed out that the inner layer of the circular muscle is also especially rich in elastic fibres.

The outer layer of the circular muscle and the longitudinal muscle of the small intestine are supplied by branches of Auerbach's plexus through the mediation of interstitial cells of Cajal. These form a very dense network in the space between the circular and longitudinal muscle layers, where Auerbach's plexus is located. They constitute a sheath to the primary and secondary plexuses of Auerbach and enter into the composition of the nerve strands. They also show definite connexions with the ganglion cells as enumerated in the previous section. The interstitial cells in this place extend into the circular and longitudinal muscles where they are connected with the finest branches of Auerbach's plexus, and supply occasional endings to the muscle. Thus together with the tertiary nerve fibres of Auerbach's plexus they form the "plexus terminal ou interstitiel" of van Esveld.

The interstitial cells are well developed here. They are mostly multipolar cells with narrow varicose processes and without differentiation into axon and dendrites. The cell body contains granules deeply stained with methylene-blue. They belong morphologically to the group of motor interstitial cells of Meyling.

My present study reveals two types of interstitial cell net: (1) located in the inner layer of the circular muscle and composed of rather primitive interstitial cells, and (2) located between the circular and longitudinal muscles and within the outer layer of the circular, composed of well-developed interstitial cells. Judging from my own observations and the facts presented by others, there seems to be no doubt about their nervous character, and that they are a

primitive type of ganglion cell. I am not in a position to say whether they can be divided into sensory and motor types. It seems to me this classification is unnecessary because not infrequently in the lower animals the primitive ganglion cells have both sensory and motor functions.

Since the inner layer of the circular muscle contains an abundance of interstitial cells which are considered as primitive nerve cells, this layer of muscle may be regarded as a neuromuscular structure.

I am in agreement, therefore, with Catel (1936) that the intestinal innervation is effected through three systems: (1) extrinsic nerves, i.e. vagus and sympathetic, (2) intramural ganglion cell systems composed of Auerbach's and Meissner's plexuses, and (3) the interstitial cells of Cajal. The first exerts a dominating influence over the other two. The last is considered as the end-part of the enteric system and is capable of carrying out the ordinary activity of the gut in the absence of the ruling influence of the other two as shown by van Esveld in ganglion-cell-free muscle preparations with physiological methods (1928*b*). That the intestinal tract maintains a primitive type of nervous organization is to be expected, since its impulses, as well as its motor activities, only involve a very simple type of nervous action. Even its rate of conduction is similar to the primitive ganglionic plexus of coelenterates (20 cm. per sec., Alvarez, 1922) and the current may run in both directions.

SUMMARY

1. The circular muscle of the small intestine of many vertebrates consists of a thick external and a thin inner layer.

2. The external layer is supplied by the tertiary branches of Auerbach's plexus and a cell net of well-developed interstitial cells of Cajal, comprising the "plexus terminal ou interstitiel" of van Esveld.

3. The inner layer is specially rich in nerve fibres which are mainly of local origin, being the protoplasmic processes of a rather primitive type of interstitial cell of Cajal.

4. The interstitial cells of Cajal are well developed in the space between the circular and longitudinal muscles and in the external layer of the circular muscle. Definite relationships between the interstitial cells, the nerve fibre strands and the ganglion cells of Auerbach's plexus, and the muscle cells are emphasized.

5. The interstitial cells of Cajal are in all probability primitive ganglion cells as suggested by Cajal, Boeke and Leeuwe.

6. Since the inner layer of the circular muscle is rich in primitive nervous elements, it may be considered as neuromuscular in nature and may have definite relations to irritability, conduction and rhythmic contractions.

7. Catel's hypothesis of intestinal innervation receives support from these observations.

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Fig. 1.

Fig. 2.

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Fig. 4.

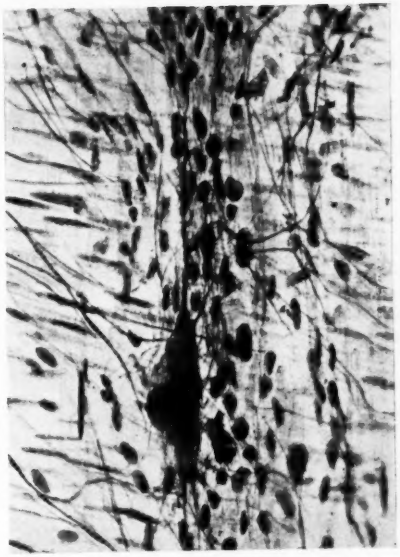
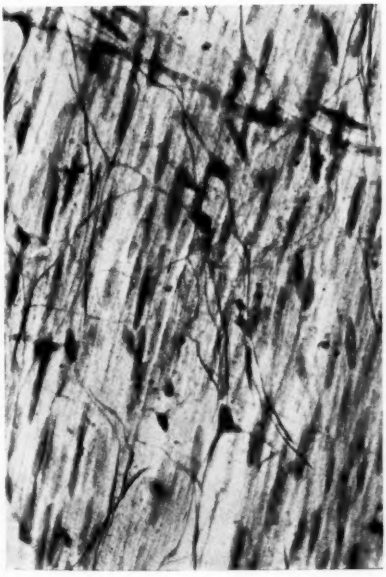


Fig. 6.



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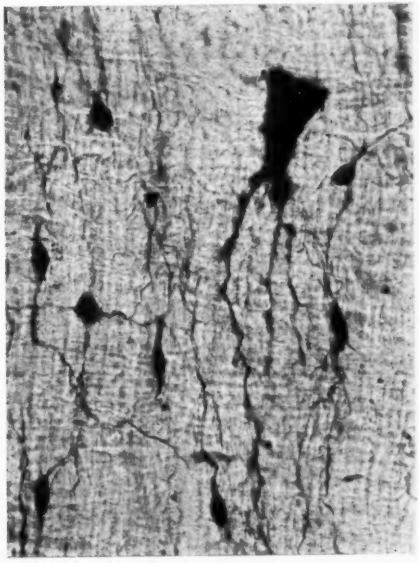

Fig. 5.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Tangential section of cat's small intestine stained by Gros-Bielschowsky method, showing interstitial cell net in the inner layer of circular muscle. The outer layer of circular muscle is clear. A, interstitial cells; B, nerve fibre; C, muscle nuclei.

Fig. 2. Rabbit's small intestine showing the interstitial cell net on the longitudinal muscle. Notice the Remak's knots at the bifurcation of fibres.

PLATE II

Fig. 3. Showing the processes of a ganglion cell and interstitial cells forming a loose network where synaptic connexions may occur. (Methylene-blue preparation.)

Fig. 4. Showing the cell body and varicotic process of an interstitial cell. (Methylene-blue preparation.)

Fig. 5. A methylene-blue preparation of longitudinal muscle showing the entwining of processes of interstitial cells and a ganglion cell.

Fig. 6. Silver preparation of longitudinal muscle of rabbit's small intestine showing the fibres of interstitial cells within and around the ganglia of Auerbach's plexus.

THE DEVELOPMENT OF THE PATELLA

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Understanding of the nature and function of the patella has acquired a new significance in view of present-day treatment of patellar fractures. It is usually considered to be a sesamoid bone, sharing the general characters of sesamoid bones, but to be outstanding among them on account of its large size, its specific and relatively constant form, and the important part it plays in the formation of the articular capsule and the mechanism of the joint to which it belongs. Its sesamoid nature and its functional importance, however, are not accepted by all. Bernays (1878) and Kazzander (1894), whose works on the knee joint are classical, and Brooke (1937) have described and illustrated the patella as developing behind the quadriceps tendon and independently of it. De Vriese (1908, 1913), on different premises, also questions its sesamoid nature and suggests that it is an integral element of the skeleton which is undergoing regression in Man. The conflicting views of the development of the patella have found a prominent place in recent clinical literature and, as part of a larger consideration of the subject, I have thought it necessary to examine a series of knee joints in embryos and young foetuses to study again the origin and early history of the patella and the manner of development of the patello-femoral joint.

The material I have used was provided for me by the staffs of several hospitals in Edinburgh and I am indebted to them for it; my special thanks are due to Dr Paterson of the Edinburgh Hospital for Women and Children. It is essential that material to be used for the study of the development of joints should be fixed without delay, for disintegrative changes quickly take place in the primitive joint-plate and false deduction of the presence of a synovial cavity may be made. Most of my specimens were immediately fixed for me in 5–8 % formalin solution.

The specimens I have examined range from the 20 mm. stage to full-term. In the smaller specimens the knee joints were isolated and serial sections made, the left limbs being cut in the longitudinal plane parallel to the flexed tibia and the right limbs in the transverse plane. In the larger specimens sections were taken at chosen intervals, and in some of them dissections were made. The usual stains were haematoxylin and eosin, but Heidenhain's iron haematoxylin and orange G and Mallory's stain were also used.

THE FORMATION OF THE PATELLA AND ITS RELATION TO THE QUADRICEPS FEMORIS

In the 20 mm. embryo, as in later stages of development, the knee joint is in the flexed position; the amount of flexion is usually about a right angle but varies a little in different specimens of the same age and often in the two limbs

of the same specimen. The femur and tibia are cartilaginous at this stage; their ends are covered with a thick perichondrio-chondrogenetic layer which consists of closely aggregated rounded cells (Pl. I, fig. 1). The posterior parts of the perichondrial zones of the two cartilages are continuous with one another but the anterior parts are separated by a distinctive layer of loose, less cellular tissue. The quadriceps femoris muscle is well represented and its lower end can be traced to the upper end of the tibia; in structure it consists throughout of a loose network, the superficial and deep surfaces of which can be defined. Where the quadriceps is in relation to the lower end of the femur there is an aggregation of rounded cells in the deeper three-fifths of its substance. This is the early representative of the patella; and, though its margins are indefinite, it is obviously confined to the substance of the quadriceps and there is a gradual transition of its cells into those of the quadriceps network. Between the quadriceps and the femur there is a layer of loose tissue which is continuous below with the loose tissue between the patella and the femur.

At the next stage examined, in the 23·4 mm. embryo (Pl. I, fig. 2), there is a considerable advance in the definition of the patella and the quadriceps muscle. The patellar aggregation is larger and more dense in structure but it still consists of rounded cells. The deeper part of the quadriceps, which contains the patella and is continuous with its substance above and below, is more differentiated than in the 20 mm. stage, but the superficial part is almost unchanged except at its lower end, over the patellar ligament, where its density is increased. On the deep surface of the patella there is a layer of loose tissue which, as in the 20 mm. stage, is continuous with similar tissue beneath the quadriceps above and in the knee joint below; there is as yet no evidence of a synovial cavity in it. The patella is seen to be developed in relation to the lower surface and the lower part of the anterior surface of the femur, and though there is no direct contact of patella and femur the lower part of the patellar surface of the femur has taken form (Pl. I, fig. 3).

The recognition of the patella at these stages of development is earlier than has been recorded by other workers. Langer (1929) describes the first appearance of the patella at the 26 mm. stage and denies its presence earlier. Bardeen (1905) does not illustrate its presence until the 33 mm. stage; and, regarding its relation to the quadriceps, he says (with reference to his figure of a 20 mm. embryo) "as the musculature becomes differentiated a dense tendon for the quadriceps is formed in front of the knee joint. In it the patella becomes differentiated". Bernays (1878) and others do not describe the presence of the patella until later stages.

The patella begins to become cartilaginous about the 30 mm. stage, but at its periphery there is still a gradual transition of its substance into the quadriceps mass which itself is more differentiated. At the 32 mm. stage (Pl. I, fig. 5) the patella is seen to be well formed and in its original position over the anterior part of the lower end of the femur. The differentiated quadriceps is continuous with its upper and lower ends and superficial to it there is an

undifferentiated layer of loose tissue which is now relatively less in thickness; it is obvious that this tissue, superficial to the patella, is in the fascial sheath of the quadriceps muscle and that below it is incorporated in the ligamentum patellae. The deep surface of the patella is separated from the perichondrial layer of the femur by the loose tissue already described in this position; there is no evidence of a synovial cavity in it and none appears in my specimens until the 35 mm. stage. Bardeen (1905) however states that a synovial cavity is present at the 30 mm. stage.

There is now a rapid increase in the growth of the patella especially in its antero-posterior thickness, and a striking change in the tissues on its deep surface. Thus at the 35 mm. stage (Pl. I, fig. 4) the formerly loose tissue between the patella and the femur is condensed to form a perichondrial layer on the posterior surface of the patella; and, further, this layer is fused with the perichondrium of the femur. The fusion of the perichondria begins over the central part of the patella and spreads to the periphery (Pl. II, fig. 7), and when fusion is complete, at or shortly after the 35 mm. stage, the patellar and femoral surfaces are united to one another by a thick perichondrium which separates the cartilages. A secondary fusion such as this must connote an absence of movement between the parts concerned and must be considered, I think, in the discussion of the determination of joint-surfaces; in effect it establishes the conditions which occur primarily in the development of the diarthroses of the axial parts of the limbs, for it leads to the definition of an articular disc or primitive joint-plate. It is not yet known if a similar developmental stage exists at the other sesamoid joints.

The thick articular disc, formed of closely packed cells, thereafter becomes reduced in thickness and the cells become elongated until, about the 40 mm. stage (Pl. II, fig. 9), only a double layer of flattened cells is present between the cartilages; these cells separate the patellar and femoral cartilages from one another so that there is no continuity of the substance either now or, in my specimens, at any later date.

The change in structure of the articular disc, it seems to me, represents the process of definition of the articular cartilages by the loss of their perichondrial coverings; and it appears to follow that the nutrition and further growth of these cartilages become functions of their deep surface and that they are established therefore as particular and distinct parts of the epiphysis to which they belong. The articular cartilages having thus been defined, the synovial cavity of the patello-femoral joint appears between them; each cartilage retains a covering of a single layer of flattened cells which is continuous at the margins of the articular area with the general perichondrium and with the articular capsule derived from it and now established. The synovial cavity appears first at the periphery on the medial side of the patello-femoral joint and then extends completely around the periphery. The extension of the cavity centrally takes place more rapidly on the medial than on the lateral side, and the fusion between the two elements persists for some time on the lateral after

their complete separation on the medial side (Pl. II, fig. 9). Langer (1929) has already noted that the formation of the synovial cavity of the knee joint is earlier and progresses more rapidly on the medial than on the lateral side. The patello-femoral cavity is at first separate from the femoro-meniscal cavity, but later communicates with it on both sides of the median septum of the knee joint which subsequently forms the infra-patellar and alar folds.

It is clear therefore that the patella retains in these later stages of development the same relation to the quadriceps mass that its primary rudiment had in the 20 mm. stage, that is, it interrupts the continuity of most of its fibres so that the vasti and rectus femoris proceed to their tibial insertion through it. The superficial uninterrupted fibres now form a perichondrium for its superficial surface and a fibrous layer over it; and these layers are continuous above with the tendon of the rectus and below with the superficial part of the patellar tendon. The patella is thus an intramuscular element which has secondarily acquired a free articular surface in a manner which is constant for the definition of the articular surfaces of movable joints, that is, by the formation of an articular disc or primitive joint-plate and its subsequent developmental change.

THE SUPRA-PATELLAR POUCH AND THE PATELLAR SURFACE OF THE FEMUR

The patella develops, it has been shown, over the antero-inferior surfaces of the femoral condyles; and it is only on these surfaces, in front of which the patello-femoral synovial cavity is formed, that the femoral articular surfaces for the patella are defined (Pl. II, fig. 8). Even before the patella has separated itself from the lateral condyle of the femur, however, the patello-femoral synovial cavity begins to extend upwards in front of the femur beyond the level of the upper border of the patella, and by the 90 mm. stage (Pl. I, fig. 6) a definite supra-patellar pouch is formed. The extension of the synovial cavity is into the tissue which lies superficial to the perichondrium of the femur; at first, before the supra-patellar pouch begins to develop, this tissue is loose mesenchyme, but later what remains of it forms the walls of the pouch and the m. articularis genu. The perichondrium on the front of the femur at the 40 mm. stage, before the supra-patellar pouch is much developed, is a thick cellular layer which extends downwards to the upper border of the articular cartilage then defined; it is shown in transverse section in Pl. II, fig. 10. As the supra-patellar pouch develops, its deep wall becomes lined with synovial membrane and fuses with the perichondrium from below upwards; thereafter the fused layer disappears and is replaced by a single layer of flattened cells (Pl. II, figs. 11, 12). Thus, the supracondylar part of the patellar surface of the femur is defined and acquires an articular cartilage. This process takes place slowly from below upwards, and is not completed until shortly before full-term; and—a striking feature—it is independent of an opposed articular surface since the articular disc, which may be considered to be formed, is constituted by the deep wall of the supra-patellar pouch with the femoral perichondrium and not by the deep surface of the patella.

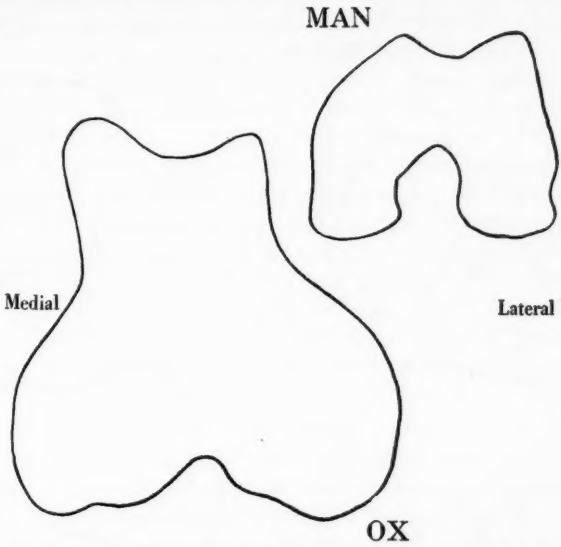
It has been shown by Langer (1929) that in embryos of the 24 mm. stage the general adult form of the bones of the knee joint already exists. I have noted further in my specimens that the supracondylar part of the patellar surface of the femur has already developed its peculiar human characters by that stage, for even then the lateral part of the surface is greater than the medial part in its transverse width, its proximal extension, and its marginal accentuation (Text-fig. 2); and this, it is to be noted, is in contrast to the delay in the development of the lateral parts of the patello-condylar and condylo-meniscal joints and in spite of the fact that throughout the greater part of foetal life at least part of the surface is covered with perichondrium. The specifically human form of the supracondylar part of the lower end of the femur is therefore primary.

This primary definition of the adult form of the supracondylar part of the femur is in strong contrast with the developmental history of the condylar part, for there the medial condyle is originally larger than the lateral condyle and, as has been shown, its articular cartilage and synovial cavity appear earlier; and it is only with the change to the characteristic adult position of the femur and with the establishment of the extension of walking, say at three years of age, that the lateral condyle becomes the dominant weight-bearing condyle and larger than the medial condyle. The femur is then directed downwards, medially, and backwards, and articulates with the tibia at an obtuse angle open laterally; and the supra-patellar part of the quadriceps and the ligamentum patellae form an almost like angle with one another at the patella. The lateral part of the patella thus becomes the chief strain-bearing part and the lateral part of its articular surface is the larger in the adult; the greater size of the lateral part of the supracondylar surface of the femur and the accentuation of its margin are the factors which maintain the patella in its proper relationship with the femur. The definition of the supracondylar surface, early and primary as it is, is thus to meet conditions which arise only well after birth.

I have found it interesting to note that in the ox—whose femur, as in ungulates generally, is directed downwards, forwards, and laterally, with the medial part of the supracondylar surface larger and more accentuated than the lateral part (Text-fig. 1)—there is the same early definition of the adult form; for in an 80 mm. ox foetus the distal end of the femur was similar in form to that of the adult animal. It follows from these observations that the essential form of articular surfaces may be defined before they are in use, that is, before movement occurs at them.

Since the patella develops in relation to the femoral condyles and passes through a stage of fusion with them, the medial and lateral areas of its articular surface are at first approximately equal in size (Pl. II, figs. 7, 9). A change in the relative size, however, begins to appear shortly after the patella has freed itself from the femur; it occurs slowly, but by the 192 mm. stage it has become established and the articular surface of the patella is then divided by a vertical ridge into larger lateral and smaller medial areas which are relatively comparable in size to those of the adult (Text-fig. 2). The change in size of these areas allows

the patella to conform to the supracondylar surface of the femur and, of course, movements permitting the apposition of the surfaces have probably occurred; but if movements are stressed as the probable cause of the changes it is necessary also to note that the upper part of the supracondylar surface is still covered with perichondrium and is non-articular (Text-fig. 2, sections C–E). The patella does not acquire the transverse ridges on its articular surface until after birth. These ridges, as was pointed out by Goodsir (1868), subdivide the surface into

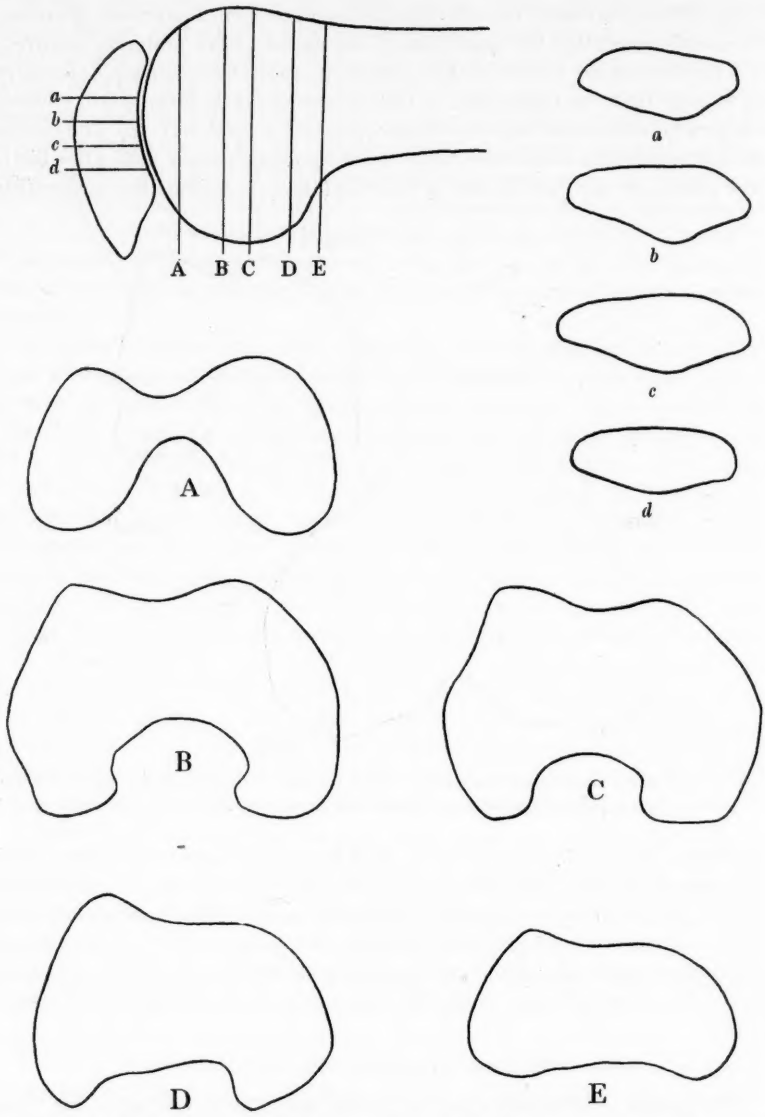


Text-fig. 1. Outline drawings of transverse sections through the supracondylar parts of human and ox femora to show the difference in form of the supracondylar articular surfaces.

functional areas which are in action in different positions of the knee joint; they appear therefore only when the limb is fully in use and full extension of the knee joint has become possible. The general and essential form of the patella is thus defined independently of the femoral surface to which it is to be opposed, but the finer particular details of the functional form are acquired only when its function is established. Such facts are probably true of the diarthroses in general.

THE SIZE AND POSITION OF THE PATELLA

The patella is relatively small compared with the distal end of the femur at its appearance and until the 35 mm. stage. Thereafter growth of the patella is rapid and, as is shown, at the 90 mm. stage (Pl. I, fig. 6) it is much larger relative to the femur. I am thus in agreement with de Vriese (1908, 1913), who has made a full study of the relative size of the patella, that it increases in relative size up to the sixth month of foetal life; she states further that until



Text-fig. 2. Projection drawings of the femur and patella of a 192 mm. foetus. The upper left figure is a projection of the femur and patella made with the tibia and quadriceps in position and the knee joint flexed to about a right angle. The outlines A–E represent transverse sections of the femur and the outlines *a–d* transverse sections of the patella at the levels indicated in the first figure. In the sections the lateral sides of the femur and patella are on the left of the page.

shortly before birth the patella grows at the same rate as the other bones of the lower limb and that thereafter and until puberty it decreases in size relative to them. I am unable to discover in these statements any evidence of retrogression of the patella.

The position of the patella on the femur in the adult varies with flexion and extension of the knee. During the early stages of its development the knee is flexed to about a right angle and the patella lies on the antero-inferior surface of the femoral condyles, a position comparable to that which it occupies in the adult when the knee is flexed to the same extent. I have not found it in my specimens in the position shown in a 33 mm. foetus by Bardeen (1905) articulating with the tibia as well as with the femur, though I have noted that in some marsupials its lower end almost touches the tibia. The patella leaves the distal surface of the femur as the knee is extended and lies more and more in contact with the supracondylar surface; and in full extension in the human subject, when the femur and tibia are in vertical alignment, it is entirely on the anterior surface of the femur. I have noted that in the ox also it lies in this position when the knee is in the position of fullest functional extension, even though there is then a considerable flexion angulation between the femur and the tibia; the patella then lies over the prominent part of the femur and it seems to me clear that in addition to its other functions it will protect the lower end of the femur from the friction of the quadriceps tendon.

SUMMARY

1. A pre-cartilaginous patella is present in the deeper part of the quadriceps mass at the level of the lower end of the femur in the 20 mm. embryo.

2. A cartilaginous patella is present at the 30 mm. stage. It interrupts the continuity of the quadriceps mass except for a superficial layer which remains undifferentiated.

3. A typical articular disc (primitive joint-plate) is secondarily formed at the 35–40 mm. stage between the patella and the femoral condyles by the fusion of the patellar and femoral perichondria.

4. The patello-femoral synovial cavity develops in the articular disc in a manner typical of the diarthroses, and with it there is a definition of the articular cartilages which are considered to be particular parts of the bones. The processes take place first on the medial side and then on the lateral side, probably reflecting the phylogenetically greater importance of the medial femoral condyle; they are completed by about the 70 mm. stage.

5. A typical supracondylar patellar surface is formed on the femur by the development of the supra-patellar pouch; it is developed independently of the patella and is not completed until shortly after birth.

6. The articular surface of the patella changes from a form adapted to the condyles to a form adaptable to the supracondylar surface soon after it is freed from the condyles. The secondary division of the patellar surface does not occur until after birth.

I desire to express my sincere thanks to Prof. J. C. Brash for his help in the preparation of this paper. I am indebted also to Mr John Borthwick for the preparation of the microphotographs.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Longitudinal section of the knee joint of 20 mm. embryo. The perichondrio-chondrogenetic zones at the ends of the femur and tibia are shown. There is an aggregation of round cells in the quadriceps mass at the level of the lower end of the femur; this is the early rudiment of the patella.

Fig. 2. Longitudinal section of the knee joint of 23·4 mm. embryo. The patellar aggregation is larger and more distinct than at the 20 mm. stage.

Fig. 3. Transverse section of the knee joint of 23·4 mm. embryo through the upper part of the patella; the lateral extension of the patella into the quadriceps mass is shown. There is some artificial separation of the loose tissues.

Fig. 4. Longitudinal section through the lateral part of the knee joint of 35 mm. foetus. The patella is larger and has acquired a perichondrium on its deep surface.

Fig. 5. Longitudinal section of the knee joint of 32 mm. foetus. The larger part of the patellar substance has become cartilage.

Fig. 6. Longitudinal section of the knee joint of 90 mm. foetus. The supra-patellar pouch extends upwards beyond the level of the patella and the m. articularis genu is attached to its apex. The patella is much increased in size.

PLATE II

Fig. 7. Oblique transverse section of the knee joint of 38 mm. foetus; the obliquity makes the patella appear thicker than it is. The patella and the femur are united by a thick articular disc (primitive joint-plate).

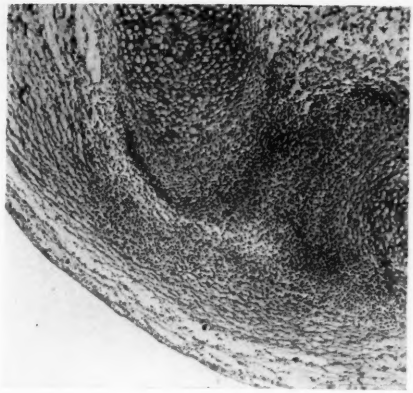
Fig. 8. Longitudinal section of the knee joint of 40 mm. foetus. The section is through the medial part of the patella and shows the synovial cavity over the medial condyle of the femur.

Fig. 9. Transverse section of the knee joint of 40 mm. foetus. The articular disc is still present on the lateral side except peripherally where the synovial cavity has appeared; on the medial side the synovial cavity is complete and the articular cartilages are defined.

Fig. 10. Transverse section of femur of same foetus as in fig. 9 above the level of the patella. It shows the supracondylar patellar surface of the femur covered with perichondrium; the lateral area of the surface is already larger and more prominent.

Fig. 11. Projection drawing of the right knee joint of 127 mm. (fifth month) foetus. The supra-patellar pouch is well formed.

Fig. 12 A and B. Transverse sections of the left knee joint of same foetus as in fig. 11 at the levels shown. A shows the supracondylar surface free of perichondrium and the articular cartilage defined, and B shows the surface covered with perichondrium. The lateral lip of the surface is the larger.



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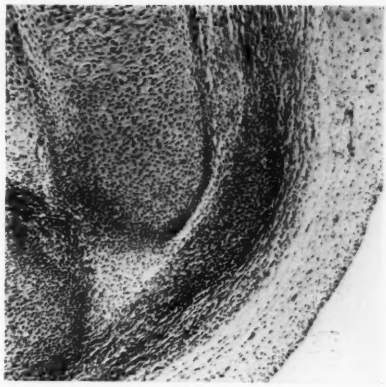

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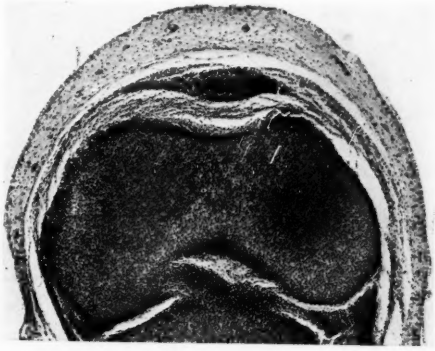

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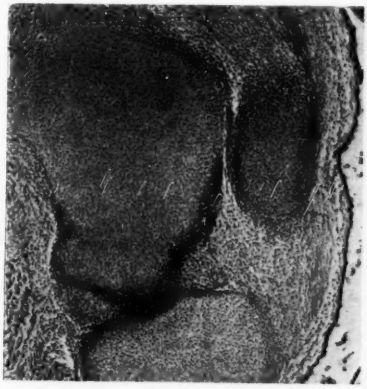

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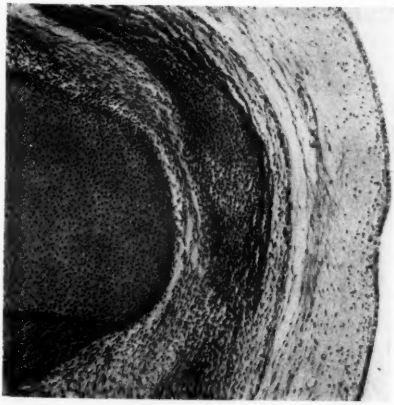

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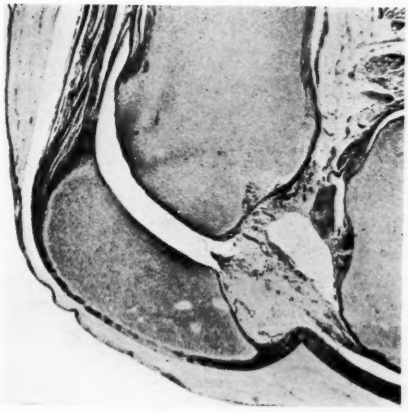

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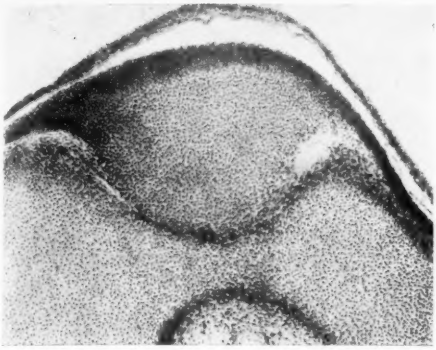

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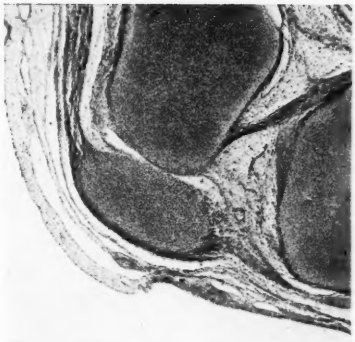

Fig. 8.

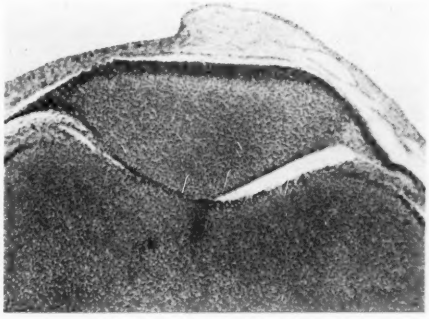

Fig. 9.

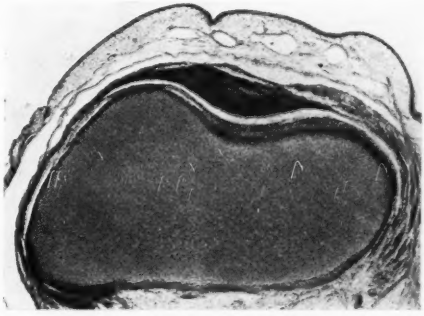

Fig. 10.

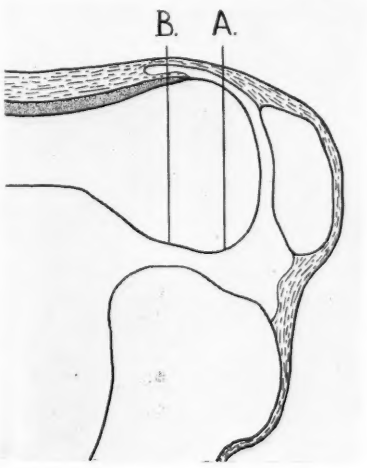

Fig. 11.

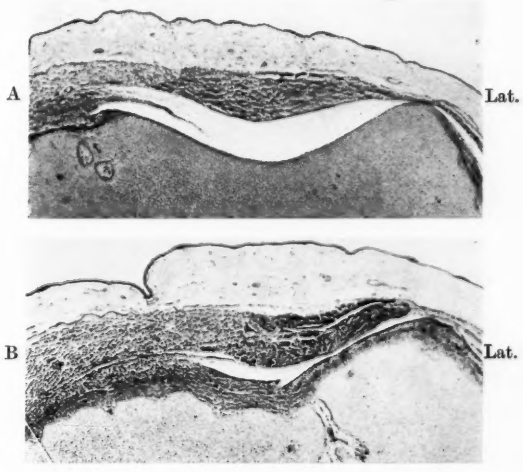

Fig. 12.

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TRANSMUTATION OF VERTEBRAE IN THE LUMBO-SACRAL REGION OF THE HUMAN SPINE

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It is now more than sixty years since Rosenberg (1875) propounded the interesting hypothesis that in man the vertebral column was undergoing a gradual process of shortening. This theory was based partly on studies of the development of the column and partly on examination of abnormalities in the vertebral column of man and of the higher apes. He considered the process to be normally ontogenetic and was convinced there was evidence that in the human foetus the 26th vertebra was the first segment to be attached to the pelvic girdle which subsequently moved forward into association with the 25th vertebra, viz. the normal first sacral. This movement headwards of the pelvic girdle on the vertebral axis was believed to indicate the trend of evolutionary change in this part of the skeletal system. In a much more recent paper the same author (1912) classified spinal variations into ten groups which in accordance with his hypothesis seemed to represent stages in the phylogeny of the human vertebral column. The human vertebral axis in its present form has twenty-four movable vertebrae anterior to the sacrum. Rosenberg considered that the ancestral form had twenty-five and that the column of the future would have twenty-three praesacral segments. The comparatively common anomaly of the partial or complete assimilation of the 5th lumbar vertebra (the 24th in the series) in the sacrum was regarded as further evidence of this evolutionary trend. Rosenberg's hypothesis did not receive much support from prominent British anatomists at the end of last century. Amongst those who contributed important papers on the subject were Birmingham, Paterson and Keith. Because of the fact that within one year amongst not more than fifty subjects two were found in which there were twenty-five movable vertebrae anterior to the sacrum or, putting it in terms of human anatomy, in which there were apparently six lumbar vertebrae, Birmingham (1891) came to the conclusion that the question as to whether Rosenberg's suggested ancestral type of man with twenty-five praesacral vertebrae occurred at the present day must be answered in the affirmative. He also expressed the opinion that if the condition be sought for it will be found to exist more frequently than is expected. On the other hand, Paterson (1893) came to the conclusion, as the result of extensive investigations on the sacrum and vertebral column, that there was not much evidence in support of the theory of the phylogenetic

shortening of the vertebral column advocated by Rosenberg. He was of the opinion that the actual variations met with might be in the direction of elongation or abbreviation of a particular region and were more often found producing elongation of the praesacral region than contraction. He also found that the great majority of the examples of variations in the arrangement of the spinal nerves in man (and the primates) indicated an extension backwards of the limb plexuses and the entrance into them of post-axially placed nerves. The evidence of the nervous system favours, therefore, the view rather of extension backwards than forwards of the lower limb in relation to the vertebral column.

From an exhaustive study of the vertebral columns of new and old world monkeys, the large apes and man, Keith (1902) came to the following conclusions regarding the evidence for phylogenetic shortening of the spine. With the evolution of the orthograde from pronograde primates, the lumbar region became relatively shorter, the process of abbreviation being brought about by the transformation of the 26th (lumbar) segment to the 1st sacral; in the evolution of the giant primates (the ancestral stock of man, the gorilla, chimpanzee and orang) the lumbar region was further shortened, the 25th segment becoming gradually sacral in character. In the origin of the human stock by the assumption of plantigrade progression, the lumbar region again became elongated and it is possible that there may be in progress a slight backward migration—a tendency for the 25th again to become lumbar in character, but the fact that the point at which sacralization commences is situated more distally in the negro than in the white man is against this assumption. In the evolution of the orang the lumbar region was further shortened, the 24th segment becoming sacral in character. In America, Dwight (1901) gave a description of a detailed study of a collection of forty-five anomalous human spines in the Warren Museum of the Harvard Medical School and discussed what is to be learned from these concerning the significance of numerical variation of the vertebrae. His conclusion that the fundamental cause of the variation is the working of "a vital principle" did not seem to add anything to existing knowledge.

That anomalous vertebral formations in the lumbo-sacral region of the human spinal column are not rare is now well known, but there seems to be little reliable information as to the frequency with which they occur in a fairly large unselected series of the apparently normal healthy population. In a recent investigation on the bony pelvis and its influence on labour which was based on a radiological and clinical study of 510 healthy young married women in London, mainly from 20–30 years of age, a fairly adequate sample of suitable material became available for an estimation of the incidence, the laterality and the probable nature of the condition as to whether it should be considered sacralization of the 5th lumbar or a lumbarization of the 1st sacral vertebra. For the interpretation of the precise nature of the abnormality we are not in the most favourable position as there are no radiographs of the

entire length of the spinal column but in the majority of cases in which the vertebral anomaly was noticed and recorded special supplementary antero-posterior and lateral radiographs of the part of the spine below the level of the lower dorsal region were taken. With the aid of these and the rather imperfect data obtained from the enumeration of the sacral and coccygeal segments sufficient evidence has been obtained to form the basis of some discussion of, and the formulation of at least tentative conclusions regarding, the nature of the anomaly. Excluding the cases in which there was merely some bulbous enlargement of the 5th lumbar transverse process and including those in which the costal element in the 5th lumbar was so expanded as to come into contact or articulate with the upper part of the sacrum there were in the unselected series of 510 women twenty-five, or 5·0 %, in which this definite abnormality in the formation of the lumbo-sacral region was clearly evident (see Table I).

Table I. *Incidence of vertebral anomalies in lumbo-sacral region of spine*

Side	Sacralization of 5th lumbar vertebra No.	Lumbarization of 1st sacral vertebra No.
Right	7	5
Right and left	14	1
Left	4	2
Total	25	8
Percentage incidence in 510 women	5·0	1·6

Of the total twenty-five, fourteen, or fully 50 %, were bilateral, seven were present only on the right side and four on the left side. In addition to the twenty-five spines showing more or less typical sacralization there were eight, or 1·6 %, of the total number which gave the appearance of a lumbarization of the 1st sacral vertebra, in so far as the upper part of the lateral mass was represented on one or other side by a process like a lumbar transverse process, and the sacrum below that level consisted of four segments. Of the eight specimens with this condition, seven showed it unilaterally, five on the right side and two on the left; one was bilateral. In Figs. 1 and 2 are outline tracings of radiographs of actual specimens showing sacralization of the 5th lumbar vertebra and lumbarization of the 1st sacral vertebra respectively.

As in a small proportion of the radiographs the lower sacral and coccygeal region was not very clearly defined it was not easy in these cases to determine with certainty where the sacrum ended and the coccyx began. In the original investigation on the pelvis, however, an attempt was made not only to enumerate the number of sacral segments throughout the series but to record in each instance the segment at the point of maximum curvature. In some pelves the line of demarcation between the first coccygeal segment and the second was more distinct than that between the former and the lower end of the sacrum, which may be evidence in support of the statement common in

anatomical text-books that the sacrum often consists of six segments having incorporated the first coccygeal vertebra. Excluding the twenty-five spines which have been described as showing sacralization of the 5th lumbar vertebra there were four specimens in which the sacrum had six segments and six in which the number was probably but not quite certainly six, i.e. ten specimens in all. Excluding the eight spines in which lumbarization of the 1st sacral vertebra has been described there were two in which the sacrum had four segments and one in which the number was probably but not certainly four; that is, three in all. The proportion of these showing probably six segments to those showing four is thus 10 : 3 or roughly 3 : 1, which is approximately the

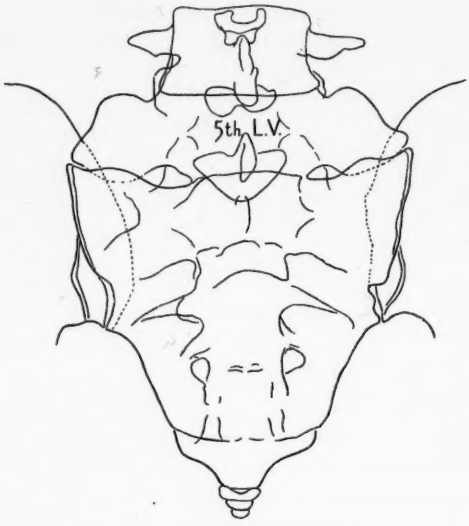

Fig. 1. Outline tracing of radiograph showing sacralization of 5th lumbar vertebra.

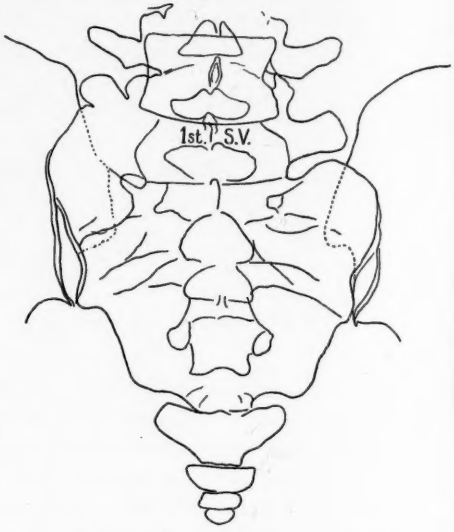

Fig. 2. Outline tracing of radiograph showing lumbarization of 1st sacral vertebra.

same as the proportion showing sacralization of the 5th lumbar vertebra to that showing lumbarization of the 1st sacral vertebra (25 : 8 or 3 : 1).

In the shorter series of fifty-two radiographs of male pelves three showed vertebral anomalies in the lumbo-sacral region so that the incidence of these seems to be of much the same order in the two sexes.

SUMMARY AND CONCLUSIONS

In a consecutive series of pelvic radiographs of 510 young married women the incidence of sacralization of the 5th lumbar vertebra was 5 %, the incidence of lumbarization of the 1st sacral vertebra 1·6 %.

While the inability to enumerate the vertebrae in the different regions of

the whole length of the vertebral columns precludes any estimate of the incidence of variations of the normal vertebral formula in these, more particularly the occurrence of six lumbar vertebrae, the fact that sacralization of the 5th lumbar vertebrae was three times more frequent than lumbarization of the 1st sacral seems to be in favour of Rosenberg's hypothesis in so far as it suggests strongly that in the series under review there is a greater tendency to shortening than lengthening of the praesacral region of the spine.

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A RADIOGRAPHIC COMPARISON OF THE MALE AND FEMALE PELVIS

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INTRODUCTION

To anatomists it has long been well known that in any collection of female pelves there are specimens in which the sex characters are so indeterminate that they may be said to resemble or even to be indistinguishable from the pelvis in the male or to be "android" in type. Two American observers, Caldwell & Moloy (1933, 8) have in recent years devoted much time to the study of the varying form of the female pelvis and its classification from the morphological point of view. Their publications have aroused considerable interest amongst obstetricians in this country, partly because of the alleged high incidence amongst females of this tendency to the male type, but mainly because of their claim to have demonstrated the importance of pelvic shape as classified by them not only in determining the type of foetal presentation but also the mechanism of labour. From the shape of the pelvic inlet judged merely by subjective impressions they have classified the female pelvis on a morphological basis into four standard or "parent" types, the "gynecoid" in which the shape of the inlet is "round" or broadly oval with the long axis transverse, the "anthropoid" in which it is oval with the long axis antero-posterior, the "android" in which it is heart-shaped, and the "platypelloid" in which it is flattened antero-posteriorly. They also classified and named intermediate or borderline forms so that in all, including an asymmetrical group, twelve categories were described. The percentage frequency of occurrence of their standard types (including the borderline forms in the nearest related standard type) amongst 147 pelves of white women in the fine collection of sexed pelves at Western Reserve University, Cleveland, U.S.A., was stated by them (1938) to be as follows: anthropoid 22·5, platypelloid 2·6, gynecoid 41·4, android 32·5. In their view about a third of the female pelves in this group thus appeared to have definite male characters and less than half were characteristically female in type. To facilitate their study of the female pelvis in the living subject they have devised a special method of taking radiographs and a special instrument known as the "precision stereoscope" as an aid to their interpretation.

In a radiological study of 215 consecutive primigravidae these authors (1934) found that, excluding intermediate forms, only 40 % were "true gynecoid" in type whereas as large a proportion as 12 % belonged to each of the

types "true anthropoid" and "true android". In view of the alleged importance of the occurrence of male tendencies in the female pelvis in influencing the course of labour it seems essential to determine the characters which are of most importance in differentiating the female from the male type of pelvis as they are shown in radiographs. A number of authors, notably Derry (1923)[1] and Strauss (1927–8), have made important contributions to the study of the characters of the female pelvis in dried skeletal material but it will be readily apparent that such detailed investigations of the characters of some of its constituent bones (the iliac) as they made are not possible in pelvic radiographs. Caldwell & Moloy (1933) in their study of the occurrence of the male or "android" type of pelvis in females have devoted most attention to the study of the pelvic inlet. From inspection, and in quite an arbitrary manner, they classify those in which it is blunt heart-shaped as "android" and those in which it is more typically female as "gynecoid". They also took measurements of some of the important diameters of the pelvis, but so far they have not published any of these. There seems now to be very general agreement for the view that classifications based on subjective impressions are not reliable. Based on personal judgments, they are too prone to be biased from one cause or another, and grading of the same characters into the same groups by different individuals, who are presumably using the same criteria, may show remarkable divergencies. When characters are measurable and expressible in numbers, the knowledge regarding them provided by this method of treatment of the data is likely to be of a much more satisfactory nature.

MATERIAL FOR PRESENT INQUIRY

In an investigation on the bony pelvis and its influence on labour, embracing a radiological and clinical study of a consecutive series of 500 women which was recently completed at the Obstetric Unit of University College Hospital and the Institute of Anatomy, University College, radiographs of the antero-posterior and lateral views of all the pelves were taken by a standardized method. From these radiographs, by appropriate adjustments for distortion, the actual dimensions of many characters of the pelvis were easily obtained. For the series of women the average values and variabilities of these characters and of certain ratios or indices derived from them were then calculated. As the best method of determining the characters in which the female pelvis differed most from the male or "android" type, as seen in radiographs from the anterior and lateral aspects, seemed to be to institute a comparison between the average values of these characters in a series of female pelves with the corresponding characters in a fairly adequate sample of typical males pelves, radiographs of the antero-posterior and lateral views of the pelvis were taken in an unselected series of fifty young adult men by exactly the same methods

[1] An attempt might have been made to calculate indices for the ilium approximately corresponding to those used by Derry and named by him the *chilotic* and the *choreumatic* had it been possible to locate the pubo-iliac point with any degree of exactitude.

as were adopted for the women. For these men the average values and variabilities of the characters under review in the women were then estimated. They are shown in comparison with the corresponding values for the women in Table I. The points from which the measurements were taken are shown in the diagrammatic outlines given in Figs. 1 and 2, which are tracings taken from photographs of the actual radiographs of the antero-posterior and lateral views of a female pelvis from the series.

TABLE I. *Showing a comparison of the mean values and variabilities of many of the principal characters shown radiographically in series of female and male pelves.*

		Females No. of observations: 375–509			Males No. of observations: 50–52		
Pelvic characters	Symbols	Means and S.E.	S.D.	Co. V. %	Means and S.E.	S.D.	Co. V. %
Obstetric true conjugate diameter	A_1B	118·3±0·44	10·0	8·5	115·1±1·30	9·4	8·2
Anatomical true conjugate diameter	AB	122·4±0·44	10·0	8·5	119·6±1·28	9·2	7·7
Greatest transverse diameter at brim	HJ	130·6±0·31	7·0	5·4	120·8±0·82	5·9	4·9
Pelvic brim index:							
(1) with obstetric conjugate	$100\,A_1B/HJ$	90·8±0·36	8·2	9·1	95·3±1·10	7·9	8·3
(2) with anatomical conjugate	$100\,AB/HJ$	93·9±0·37	8·3	8·9	99·2±1·07	7·7	7·8
Approximate area of pelvic inlet:							
(1) with obstetric conjugate	$\pi HJ \times A_1B/4$	121·6±0·58	13·2	10·8	109·5±1·69	12·2	11·1
(2) with anatomical conjugate	$\pi HJ \times AB/4$	126·8±0·66	12·8	10·1	113·4±1·72	12·4	10·9
Antero-posterior diameter at pelvic outlet	A_2C	119·7±0·44	8·6	7·2	119·4±1·21	8·6	7·2
Transverse diameter at outlet (interspinous	ab	99·5±0·37	7·1	7·1	82·9±0·85	6·2	7·4
Intertuberal breadth	EF	109·0±0·32	7·0	6·4	92·9±0·89	6·4	6·9
Approximate area of pelvic outlet	$\pi ab \times A_2C/4$	93·7±0·54	10·4	11·1	78·0±1·25	8·8	11·3
Subpubic angle	$\angle R_1A_2R_2$	93·5±0·34	7·4	7·8	75°8±0·81	5·8	7·6
Sacro-sciatic notch index	$100\,VW/K'L$	52·4±0·37	7·6	14·5	61·8±1·08	7·8	12·6
Index of pelvic funnelling	$100\,(HJ-EF)/QR$	21·2±0·28	6·2	29·0	24·4±0·89	6·4	26·1
Index of sacral curvature	$100\,EF/BC$	18·3±0·28	6·2	33·9	21·9±1·03	7·4	33·7
Sacral index	$100\,XY/BC$	108·9±0·43	9·6	8·8	109·8±1·52	10·8	9·9
Sacral inclination to pelvic brim	$\angle ABC$	76°4±0·29	6·4	8·3	74°4±0·79	5·6	7·6
Sacral inclination to pelvic brim	$\angle ABF$	92°6±0·38	8·5	9·1	93°5±1·08	7·7	8·2
Inclination of pelvic brim to horizontal plane	$90°-\angle DAB$	57°7±0·28	6·1	10·6	57°2±0·78	5·6	9·9
Posterior sagittal segment ratio at inlet	$100\,BZ/AB$	48·7±0·34	7·1	14·5	46·6±0·99	7·0	15·0
Ilio-innominate ratio	$100\,MO/MX$	65·7±0·20	4·5	6·9	66·7±0·53	3·8	5·7
Posterior sagittal segment ratio at outlet	$100\,K'C/A_2C$	33·0±0·23	4·8	14·5	25·7±0·67	4·8	18·6

Units of measurement. The means and standard deviations of the linear measurements are in mm., of the areas in sq. cm., of the angles in degrees and of the ratios or indices in percentages.

METHODS OF MEASUREMENT OF PELVIC CHARACTERS

Before comparing the mean values and variabilities in the male and female series it may be advisable to refer briefly to some of the characters and the method of measuring them which was used. Their exact nature will be made more apparent from the lettering of the figures and the symbols used to denote

them in Table I. There are two true conjugate diameters on the list, the anatomical true conjugate and the obstetric true conjugate. These are measured on the lateral radiograph from the sacral promontory to the posterior margin of the upper end of the symphysis pubis and to the summit of the eminence on the posterior surface of the symphysis pubis a little below its upper end respectively. The anatomical conjugate is on the average 3 or 4 mm. longer than the obstetric. The greatest transverse diameter at the brim is found by trial on the antero-posterior radiograph. Two values of the pelvic brim index (100 × conjugate diameter/greatest transverse diameter at brim) have been calculated, one using the obstetric, the other the anatomical conjugate. At the outlet of the bony pelvis, the measurement in the antero-posterior direction is taken on the lateral radiograph from the lower border of the symphysis pubis

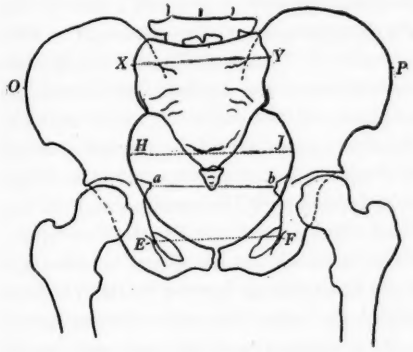

Fig. 1. Outline tracing of radiograph of the antero-posterior view of the pelvis showing the principal measurements taken.

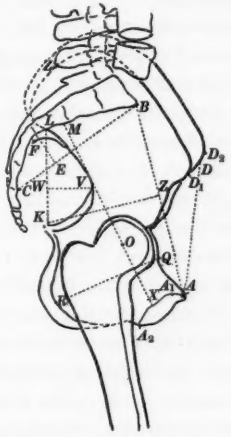

Fig. 2. Outline tracing of radiograph of the lateral view of the pelvis showing the principal measurements taken.

to the lower end of the sacrum at the sacro-coccygeal junction; in the transverse direction two measurements are taken from the antero-posterior radiograph, the interspinous diameter or the distance between the extremities of the ischial spines and the intertuberal breadth which is the distance between the inner margins of the ischial tuberosities. The approximate areas of the pelvic inlet and outlet have been calculated from the antero-posterior and transverse diameters on the assumption that the openings are approximately elliptical in shape and that the major ($2a$) and minor ($2b$) axes of the ellipse can be represented by these diameters. The formula πab then gives the area. Comparison of the actual measurement of the inlet areas in a number of radiographs by the planimeter with the corresponding areas obtained by using the diameters as the elliptical axes in which the agreement is usually fairly close seems to give adequate support for the validity of the assumption. The width of the

subpubic angle in the individual pelves was obtained from the ratio of two chords, half the intertuberal breath and the height of the pubic arch. The former was measured on the antero-posterior radiograph as already described, the latter measured on the lateral radiograph is the distance from the lower margin of the symphysis pubis to the lower border of the ischial tuberosity. Both having been adjusted by the use of correcting factors for distortion, the ratio gives the cotangent of half the subpubic angle. From a table the values of the corresponding angles can easily be obtained.

The sacral index is 100 × straight sacral breadth/straight sacral length, the components being obtained from measurements on the two radiographs adjusted for distortion. The index of sacral curvature is 100 times the ratio of the subtense from the deepest point of the concavity of the sacrum to the straight sacral length over the straight sacral length. The inclination of the sacrum is measured by two angles, *ABC* and *ABF*, on the lateral radiograph. These are the respective angles which the true conjugate diameter, *AB*, makes at the sacral promontory with (*a*) the straight sacral length, *BC*, and (*b*) the chord from the sacral promontory to the deepest point of the sacral curve, *BF*.

The descriptive terms "narrow", "wide", "gynecoid", etc. being considered very unsatisfactory for a classification of the sacro-sciatic notch, an attempt was made to obtain a numerical index of its form in the following way. On the shadow of the notch nearest the film on the lateral radiograph a chord was drawn from the base of the ischial spine to the point where the lower margin of the ilium bounding the notch behind met the sacrum, and on this chord a perpendicular was dropped from the deepest point of the concavity of the notch. The ratio of 100 times this subtense to the chord on which it stands is the sacro-sciatic notch index: its size bears an inverse relation to that of the notch, or in other words, the higher the index the narrower the notch. The index shows a considerable range of variation in both the male and female series and, though it cannot be claimed to be an accurate criterion, it does bring out differences in the form of the notch in each sex group. The amount of convergence in the side walls of the pelvis or pelvic funnelling was measured by an index which took the form: 100 (greatest transverse diameter at the brim—intertuberal breadth)/depth of the pelvis from the ilio pectineal eminence to the lower border of the ischial tuberosity on the side adjacent to the film. As it has been stated that one of the features in which the female pelvis differs from the male is in the extent of iliac bone that intervenes between the acetabulum and the auricular surface for articulation with the sacrum, the female with the wider sacro-sciatic notch being relatively the larger in this respect, the difference between male and female almost suggesting that the female type could be produced from the male by the insertion of a short segment of bone at the summit of the sacro-sciatic notch, an attempt was made to measure this character by a ratio which was devised and will be referred to as the ilio-innominate ratio. It was obtained in the following way. On each lateral radiograph the centre of the acetabular cavity on the side of the pelvis

adjacent to the film was clearly marked and through this point, *O*, a straight line was drawn passing backwards just above the summit of the sacro-sciatic notch till it met the sacrum at *M* and forwards till it met the shadow of the pubic ramus at *X*. $100\,MO/MX$ is the criterion used. Another ratio on the list which is referred to as the posterior sagittal segment ratio at the pelvic inlet should be a guide to variations in the same feature of the pelvis. Caldwell, Moloy & D'Esopo (1934) state that, when the pelvic brim is held in the horizontal plane, in all their pelvic forms the widest transverse diameter at the brim lies in the same coronal or frontal plane as the ischial spines. At the point of intersection of the true conjugate and transverse diameters of the pelvic brim the former is divided into a posterior and an anterior sagittal segment. On the assumption that the relationship described by Caldwell & Moloy held fairly approximately in our series of women and men, on each lateral radiograph a perpendicular was dropped from the mid-point between the ischial spines to the true conjugate diameter. The segment of the true conjugate diameter behind the point of intersection was taken as a percentage of its total length to give the index. This ratio like the ilio-innominate ratio should indicate variations in the "depth" or relative extent of the posterior sagittal segment of the pelvic inlet. The posterior sagittal segment ratio at the pelvic outlet is a similar ratio obtained from the pubo-sacral or antero-posterior diameter at the outlet on the lateral radiograph by dropping a perpendicular on it from a point midway between the bases of the ischial spines.

The inclination of the pelvic brim to the horizontal plane was measured in the following way. On each lateral radiograph, by a special goniometer with long arms, the angle which the anatomical true conjugate continued to a point on the front of the symphysis pubis made with the line passing from the latter point to the mid-point of a line connecting the tips of the anterior superior iliac spines, was measured and recorded. In the living subject in the erect position the three points, the front of the symphysis pubis and the tips of the two anterior superior iliac spines, are said to lie approximately in the same perpendicular coronal plane, the spino-symphysial plane. In the individual members of our series of men and women when standing erect the deviation of the spino-symphysial plane from the vertical was measured by a simple instrument which was devised for the purpose. This consisted of a wooden framework of three adjustable arms supporting small vertical plates to be applied to the iliac spines and front of the symphysis respectively and carrying on a projecting rod, at the point of intersection and at right angles to the arms, a detachable goniometer from the graduated scale on which the amount of deviation of the plane from the vertical was easily read and recorded. In 473 women the range of variation from the vertical was from plus 10° to minus 10°, but 36 % were exactly vertical and the average deviation from the perpendicular was only 1°. In fifty-two men the range of deviation was the same as in the women; in 70 % the plane was exactly perpendicular and the average deviation was less than 0·5°. For the individual pelves the angles, if any, giving

the deviation in a plus or minus direction of the spino-symphysial plane from the vertical were added to or deducted from the angle shown in the lateral X-ray giving the inclination of the true conjugate to the spino-symphysial line. The resulting angles were the angles of inclination of the true conjugate (or approximately the pelvic brim) to the vertical plane. The complement of each angle, or the difference from 90°, gave the inclination of the pelvic brim to the horizontal plane.

COMPARISON OF CHARACTERS IN MALE AND FEMALE PELVES

Size and proportions of pelvic inlet

As is shown in Table I the characters amongst those analysed which appear to be most important in differentiating the male and female pelvis are the size and proportions of the pelvic inlet, the size and proportions of the pelvic outlet, the size of the sacro-sciatic notch and the size of the subpubic angle. Of less importance are the amount of convergence of the side walls of the pelvis and the degree of sacral curvature. Both the diameters at the pelvic brim, the true conjugate and the greatest transverse, are on the average significantly less in the male than in the female pelvic series, but whereas the true conjugate is only some 3 mm. less, the transverse diameters show a difference of 10 mm. or three times as much. Associated with the smaller diameters the mean area of the pelvic inlet in the males is considerably less than that in the females. The difference is as much as 12 or 13 sq. cm. As the greatest transverse diameter at the brim in the male pelvis is relatively more diminished than the true conjugate in comparison with the corresponding diameters of the female pelvis, the ratio of the true conjugate diameter to the transverse diameter or the pelvic brim index is greater in the former. The difference is about 5 units using either conjugate diameter. While the pelvic brim index undoubtedly indicates the general shape of the pelvic inlet, viz. as to whether it tends to be circular or is oval with the major axis in the sagittal or transverse directions and the degree of flattening in these planes, it does not, as pointed out by Caldwell & Moloy, differentiate the wedge-shaped or "android" type of inlet. In the series of pelves examined the shape of the inlet is undoubtedly more nearly circular on the average amongst the males than the females, though the typical female pelvic inlet is often somewhat loosely referred to as "round" in form. A similar difference in the pelvic index of men and women to that shown has been described for other racial types, including the Australian, Negro, Andamanese etc. (see Deniker, 1900). It must be pointed out that these average measurements of the diameters at the inlet derived from radiographs of the living are not in very close agreement with the corresponding average diameters recorded in anatomical text books which have probably been estimated in the majority of cases from dried pelves. Thus Bryce in Quain's *Anatomy* (1915) quotes the average transverse diameter and conjugate diameter at the pelvic brim in full-sized females as being 133 and 134 mm.

respectively, which give a pelvic brim index of 86 approximately. In full-sized males the corresponding diameters are said to be 127 and 102 mm., giving an index of 80 approximately. These values would suggest that the male pelvis is relatively more elongated in the transverse direction or flatter than the female, a view which is completely at variance with the relationship found in the series of Londoners and in many other racial types. There is strong reason for the belief that the average dimensions now recorded and accepted for the diameters of the pelvis, more especially the true conjugate, are not truly representative of those that are found in the pelves of English men and women of the present day and are in need of careful revision. As radiographs showing the view of the pelvic inlet parallel to the film were not taken, we are not in the most favourable position to comment on Caldwell & Moloy's observations or to institute a direct comparison between their results and ours. Even from the radiographs showing the antero-posterior views of the pelvic inlet it is possible to select some that appear to be of "android" type, viz. those showing a definite tendency to be blunt heart-shaped with the greatest transverse diameter at the brim located relatively far back near the base of the triangle. The frequency of occurrence of this type of inlet in our series of female pelves cannot be compared with that found in Caldwell & Moloy's series because the criteria on which classification of types is based, being merely personal impressions and incapable of standardization, are not the same. It may be of interest to mention, however, that in the series of about 500 female pelves forty or 8 % seemed to have a definite android appearance. In a further thirty or 6 % the android tendency was less clearly and sometimes rather doubtfully indicated. Thus in our view the total incidence of the android type in the series would not be higher than 14 %, i.e. less than half the incidence found by Caldwell & Moloy. In the android group of forty pelves the mean size of the sacro-sciatic notch was rather smaller than in the whole series but the size of the subpubic angle did not differ. These two characters, as has been mentioned, are amongst those of greatest value in distinguishing the female from the male type of pelvis. It is worthy of note that in the male series of fifty pelves there are some which show a typically gynaecoid form of inlet.

Size and proportions of pelvic outlet

While the mean antero-posterior diameter at the pelvic outlet, measured from the lower border of the symphysis pubis to the lower end of the sacrum at the sacro-coccygeal junction, is the same in the female and male series of pelves the transverse diameters in this region, both interspinous and intertuberal, are much smaller on the average in the male, the mean sexual difference being as much as 16 mm. (Table I). As a consequence of the relatively great narrowing of the elliptical outlet in the male, its area, as estimated from the pubo-sacral and interspinous diameters, is much less in the latter than in the female, the mean difference being about 16 sq. cm. The average values given for the antero-posterior or conjugate diameter at the outlet of the female

and male pelves given in Table I, 119–120 mm., differ greatly from the similarly designated diameter for adult pelves quoted by Bryce in Quain's *Anatomy*, 108 mm. for the female and 85 mm. for the male. The average values given for the intertuberal breadth in Table I, 109 mm. for the female and 93 mm. for the male, also differ greatly, especially the former, from the corresponding averages quoted in Quain for the transverse diameter at the pelvic outlet, viz. 141 and 88 mm. The measurements may not have been taken from exactly the same anatomical points but it is impossible to give any reasonable explanation for differences of the magnitude observed. As with the diameters at the pelvic inlet the values for the mean dimensions of the pelvic outlet which have been more or less generally accepted by anatomists hitherto appear to be in much need of verification if not revision.

Subpubic angle

The mean values given for the size of the subpubic angle in the male and female series of pelves are not comparable with the corresponding values published by other observers as they have not been estimated in exactly the same way. As measured by the method used, however, the angle clearly indicates the definite sexual difference in the size of this character, its mean value being about 18°.

The sacro-sciatic notch index

In the female series the average value of this index is 52, in the male 62 approximately (Table I). As the larger the index the smaller the notch, the latter is clearly of a narrower and deeper form on the average in the male. The numerical method adopted of estimating the size of the notch is much more reliable and informative than the use of descriptive terms such as narrow, U-shaped, wide, gynaecoid, etc., based on personal impressions.

Other pelvic characters

The amount of convergence in the side walls of the pelvis in passing from the inlet to the outlet and the degree of curvature of the sacrum are on the average significantly greater in the male than in the female pelvis. As well as being flatter than in the male the female sacrum is usually said to be relatively broader in proportion to its length. This relationship is not shown in the series of pelves under review, the sacral index is of the same order in both sexes. While the mean sacral index for the group of males in Table I, 110, is approximately the same as that usually quoted for the male sex, 112 (see Quain), the mean value for the female, 109, is as much as 7 units less than the mean sacral index (116) given for the female in Quain. It is thus only in the females of our series that the proportion of breadth to length of the sacrum seems to deviate from that found on the average and recorded for specimens from Europeans. As the sacrum is relatively longer the index tends towards the value found for the more primitive racial type, the negress.

The angle of inclination of the pelvic brim to the horizontal is usually stated to be slightly greater in the female than in the male; the same sexual relationship is said to hold for the sacro-pelvic angle (*ABF*) which the pelvic brim makes with the front of the upper part of the sacrum. In Table I the mean values given for these two angles do not differ in the two sexes. The angle which the pelvic brim (true conjugate) makes at the promontory with the straight sacral length ($< ABC$) is on the average 2° greater in the female than in the male. The difference, though relatively small, is statistically significant and is probably mainly dependent on the greater longitudinal curvature of the male sacrum.

The ilio-innominate ratio, which it was hoped would bring out the relatively greater extent of the part of the os ilium between the sacrum and acetabulum in the female pelvis than in the male, does not differ in the two sexes (as can be seen from Table I). The posterior sagittal segment ratio at the inlet which might be expected to give some indication of any difference in relative growth in the same region of the ilium is higher by 2 units in the female than in the male. The difference is significant statistically but seems to be of too small an order to be of much practical importance in differentiating the male from the female pelvis. The posterior sagittal segment ratio at the pelvic outlet is distinctly greater in the female than in the male (Table I). The sexual difference is probably largely dependent on the greater inclination of the sacrum to the pelvic brim and the greater flattening of the sacrum in the female.

TABLE II. *Showing the correlation coefficients between a selection of pairs of pelvic characters*

Variables		r
Pelvic brim index and sacro-sciatic notch index	(424)	0·132 ± 0·048
Pelvic brim index and size of subpubic angle	(469)	0·005 ± 0·046
Subpubic angle and sacro-sciatic notch index	(422)	0·142 ± 0·048
Subpubic angle and area of pelvic inlet	(360)	0·018 ± 0·053
Subpubic angle and area of pelvic outlet	(360)	0·122 ± 0·052
Area of pelvic inlet and area of pelvic outlet	(375)	0·504 ± 0·039

DISCUSSION

The pelvic characters amongst those shown and measurable in the antero-posterior and lateral radiographs which seem to be of most value in differentiating the male from the female pelvis, and which might be expected by analogy to distinguish the android type from the more typical form of female pelvis, have been described. They are the size and proportions of the pelvic inlet and outlet, the size of the subpubic angle and the size of the sacro-sciatic notch. If films of the pelves showing the outline of the inlet parallel to the horizontal plane had been available it would have been possible, by the use of chords and the arcs obtained by dividing its contour into segments, to devise some numerical expression describing its form in detail which would have been much more precise and informative than the use of purely descriptive

terms, android, gynecoid, etc. There appears to be a surprisingly small degree of association between the different pelvic characters, or for a pelvis which is "more female" in type in one character to show the same tendency in another. In the female series of nearly 500 the relationships between numerous pairs of pelvic characters were estimated by the method of correlation. A small selection of the coefficients of correlation which were calculated are shown in Table II. There is a moderate degree of correlation between the areas of the pelvic inlet and outlet ($r=0{\cdot}5$). A slight but statistically significant tendency is present for the sacro-sciatic notch to narrow as the pelvic brim becomes relatively elongated transversely ($r=-0{\cdot}132$), for the notch to increase with increase of the subpubic angle ($r=-0{\cdot}142$) and for the subpubic angle to increase with increase in area of the pelvic outlet ($r=0{\cdot}122$), but as the coefficients are all less than 0·15 in value the degree of association indicated cannot be considered of much practical importance. There is no correlation whatever between the shape of the pelvic brim and the size of the subpubic angle nor between the area of the pelvic inlet and the size of the subpubic angle.

As may be seen in Table I there is a fairly close agreement between the variabilities of the corresponding characters in the male and female series of pelves. The variation shown in most of them is considerable, the coefficient of variation being often nearly 10 %. In the area of the female pelvic inlet this variability corresponds to a range of from 87 to 165 sq. cm. In the ranges of the frequency distributions of the several pelvic characters which differ significantly in their mean values in male and female series of pelves there is a very considerable overlap and there are relatively few values of any one character in the one sex that may not be found at least occasionally in the other. Strauss (1927–8) found a considerable range of dispersion and sexual overlapping in all the characters of the ilium which he examined. He came to the conclusion that "sexing unknown pelvic material borders upon guesswork". While many anatomists will perhaps support his inference that individual characters, because "they vary so greatly and exhibit such marked sexual overlapping, are of limited value in sexing the pelvis" most will maintain there is sufficient evidence to warrant the view that from a consideration of a combination of characters it is possible to be fairly confident of distinguishing the sex in all except a small proportion of pelves.

SUMMARY AND CONCLUSIONS

A detailed comparison has been made between the means and variabilities of a large series of characters which have been measured in antero-posterior and lateral pelvic radiographs of unselected groups of men and women with the object of determining which of these characters are of most importance in distinguishing the male pelvis or the so-called "android" type of female pelvis from the more typical form of female pelvis. There seems to be sufficient evidence to warrant the following conclusions.

1. Of the characters brought under review, those that seem to be of most importance in distinguishing the male from the female pelvis, and by analogy the "android" type from the more typical form of the female pelvis, are the size and proportions of the pelvic inlet and outlet, the size of the sacro-sciatic notch and the size of the subpubic angle.

2. Of less importance but still of value are the degree of convergence of the side walls of the pelvis or pelvic funnelling and the degree of curvature of the sacrum.

3. The variabilities shown by corresponding characters in the two sexes are usually in close agreement and are fairly high, being often about 10%.

4. The two characters measurable in the radiograph, the ilio-innominate index and the posterior sagittal segment ratio at the inlet, which might be expected to bring out in the female the relatively greater extent of iliac bone between the acetabulum and the auricular surface for articulation with the sacrum, do not differ very appreciably on the average in the two sexes.

5. With one or two exceptions, e.g. the areas of the pelvic inlet and outlet, the correlation between any pair of pelvic characters is usually of a small order and of no practical importance.

6. The characters thus seem to vary in size or form more or less independently of one another. This relative lack of association is found even between such definite sexual characters as the subpubic angle and the sacro-sciatic notch.

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THE FIBRE COMPONENTS OF THE LARYNGEAL NERVES OF *MACACA MULATTA*

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The structure and functions of the laryngeal nerves of mammals have been investigated by Burkart, Krause, Russell, Hembecker & O'Leary, Arione, Rubaltelli and Du Bois & Foley.

Our investigations have been carried out on specimens of *Macaca mulatta*, partly on intact animals and partly on one in which the roots of the left vagus nerve and its radix accessoria s. bulbar accessorius had been divided (by R. J. B.) proximally to the jugular and nodose ganglia 33 days previously, to allow of degeneration of the motor fibres in the laryngeal nerves.

The results, for the most part, are similar to those of Du Bois & Foley, but differ in some particulars and give additional information on the sensory nerve endings (muscle spindles) in the crico-thyroideus and the laryngeal muscles.

It was first found that in intact animals the number of medullated fibres is about equal on the two sides, but varies a little from one individual to another. These fibres are of various sizes from about 1·5 μ in diameter to various maxima, as stated below. All branches also contain non-medullated fibres. The branch of the left r. laryngeus superior to the crico-thyroideus in the vagotomized animal contains 102 healthy medullated fibres, of various sizes up to 10 μ in diameter. On the right (normal) side 334 fibres of various sizes up to 10 μ in diameter are visible. In an intact animal 287 fibres were found on the left side and 276 on the right. It follows that in this branch about 70 % of the medullated fibres are motor and 30 % muscle-afferent s. proprioceptive. Muscle spindles are present on both sides.

This percentage of muscle-afferent fibres passing to the crico-thyroideus is similar to that found by Sherrington (1894–5) in the nerves to limb muscles in the cat and *Macaca mulatta*. One of us also found (1913) that in *Macaca irus* 34–36 % of the medullated fibres of the Vth cranial nerve passing to the muscles it innervates are afferent. The r. laryngeus recurrens for the major part of its course consists of two constituents, laryngeal and sensory, surrounded by a common sheath (Fig. 1). On the left side of the vagotomized animal the laryngeal constituent consists, for the most part, of degenerated fibres. The sensory constituent, of normal fibres of various sizes up to 8·5 μ in. diameter, is crescent shaped and forms about a third of the nerve. It diminishes in size towards the larynx owing to its giving off fine branches to the oesophagus and trachea. It ends by becoming continuous with the communicating sensory branch of the r. laryngeus superior.

The laryngeal constituent separates from the sensory constituent near the larynx and then divides into two branches which pass to the abductor and adductor muscles (Fig. 2). Six non-degenerated fibres, of various sizes up to 10 μ, are present in the branch to the abductor muscle and eight in that to the adductor muscles. On the right (normal) side 184 medullated fibres, of various sizes up to 10 μ in diameter, are visible in the branch to the abductor muscle and 297 in that to the adductor muscles.

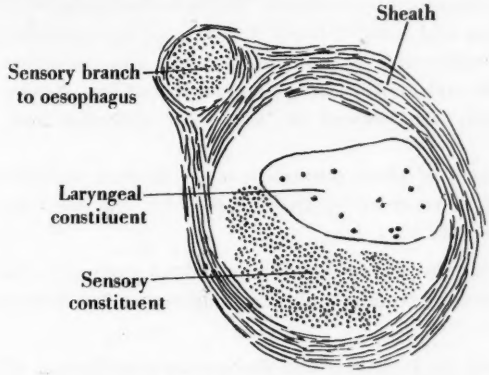

Fig. 1. Right recurrent laryngeal nerve. The laryngeal constituent contains 14 normal fibres; the others are degenerated. × 100.

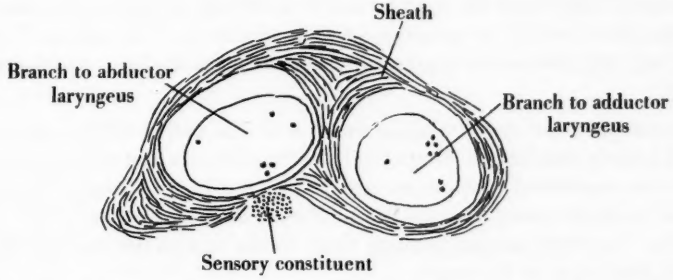

Fig. 2. Right recurrent laryngeal nerve, upper part. The branch to the abductor larynges contains six normal fibres, that to the adductor muscles eight normal fibres. × 100.

It follows that about 97 % of the medullated fibres in the laryngeal constituent are motor and 3 % muscle-afferent s. proprioceptive. In accordance with these observations sensory nerve endings (muscle spindles) are present, though scanty, in the laryngeal muscles of both sides.

The three fasciculi depicted in Fig. 2 are homologous with those found by Russell (1892) in the dog, one passing to the adductor muscles, the second to the abductor muscle, and the third "non-motor" in function. In that animal, however, separation of the fasciculi, "possible by a thin-bladed knife",

extends back in the neck, and it was possible to stimulate each individual fasciculus.

In man the maximum diameters of both the motor and sensory medullated fibres are the same, 10 μ. The branch of the r. laryngeus superior to the crico-thyroideus contains 558 medullated fibres. Distal to that branch the nerve contains about 900 fibres. In three subjects, the whole length of the r. laryngeus recurrens in the neck was found to be separated into from ten to twelve fasciculi each surrounded by a sheath. Owing to the similarity in the maximum size of the motor and sensory fibres it could not be determined which of the fasciculi were motor and which sensory,[1] but one fasciculus passed to the abductor muscle and another to the adductor muscles. Scanty nerve endings (muscle spindles) are present in both the abductor and the adductor muscles.

On comparison of these observations on *Macaca mulatta* and man with those of the cat, as recorded by Du Bois & Foley, the following differences are found to be present:

(1) In the cat the sensory medullated fibres passing to the mucous membrane of the larynx, trachea and oesophagus and the laryngeal muscles are all small.

(2) In the cat the laryngeal and the sensory constituents of the r. laryngeus recurrens remain in a common sheath right up to the laryngeal muscles.

(3) In the cat Du Bois & Foley stated that "few, if any, of the small myelinated fibres enter the muscles and it is difficult to believe that they play any significant role in the proprioceptive innervation of the larynx. It would seem that they are nerves of general sensation to the trachea, oesophagus and larynx."

Examination of the laryngeal muscles of the cat, however, shows that scanty muscle spindles are present in both the abductor and adductor muscles. It is to be concluded that the recurrent laryngeal nerve of man, *Macaca* and the cat contains scanty muscle-afferent fibres passing to muscle spindles in the muscles. Impulses passing through them to the central nervous system take part in regulation of the voice.

The reflex effects found by Burkart (1868) and Krause (1892) by stimulation of the central end of the divided recurrent laryngeal nerve are probably due to the sensory constituent of the nerve.

The motor nerve endings in the crico-thyroideus and the laryngeal muscles of many mammalian genera were described by Arione in 1924.

Part of the cost of this investigation was defrayed by the Colston Research Society.

[1] Rubaltelli (1934) showed in human foetuses that fine sensory branches penetrate to the submucous tissue of the trachea and oesophagus.

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THE COMPARATIVE MORPHOLOGY OF THE PLATYSMA

A COMPARATIVE STUDY OF THE SPHINCTER COLLI PROFUNDUS AND THE TRACHELO-PLATYSMA

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THE SPHINCTER COLLI PROFUNDUS AND THE TRACHELO-PLATYSMA

Though Ruge considered the extensive platysma of the Cercopithecidae and anthropoids to be derived from a single matrix, he suggested its subdivision into a Nacken-platysma, whose fibres took origin dorsal to the point of the shoulder, and a Hals-platysma, which took origin from the front of the chest. To avoid the hybrid words the terms noto- and trachelo-platysma have been suggested for these two subdivisions of the platysma (Lightoller, 1928). The derivation of the noto- and trachelo-platysma from a common matrix has many objections: amongst them may be mentioned the very great size and wide distribution of the total sheet; the late appearance, phylogenetically, of the trachelo-platysma, and that its appearance, in Placentalia, is always accompanied by the loss of the sphincter colli profundus. Also that the trachelo-platysma gradually replaces the noto-platysma (Lightoller, 1928); and there is noticeable, at times, a definite difference in the texture and colour of these two portions of the platysma. In these cases the trachelo-platysma appears more delicate in character and paler in colour than the noto-platysma.

Recently (Lightoller, 1939) it has been suggested that the trachelo-platysma is not derived from the noto-platysma, but from the sphincter colli profundus. This suggestion was based upon the observation that a full development of the sphincter colli profundus and the trachelo-platysma never occurred in the Placentalia, though both were present in marsupials. In the latter, it was noticed that the fibres of the one muscle ran in the same direction as, and a proportion were continuous with, those of the other. This was demonstrable even when the two muscles were inseparable.

In the following description three phylogenetic stages will be described:

A. *Sphincter colli profundus.* In its simpler and more usual form this sheet is found in the lower mammalia and lesser primates (Fig. 1 A); it has been so adequately described by various authors that a brief summary of its morphology will suffice here. It is a large sheet of muscle which takes origin in the mid-ventral plane by interlacement with the corresponding fibres from the opposite side. The origin may be an extensive one, extending from the middle or lower end of the sternum to the mentum. From their origin the fibres pass

at first laterally, then dorsally to cover the side of the neck and face. In this region it lies deep to the noto-platysma. For convenience of description and reference the sheet has been divided into five portions: P.p. cervicalis, auris, intermedia, palpebralis and oris. A point to which very little attention has been paid is the marked contrast between the rostral and caudal fibres. The rostral fibres are transverse in direction, and often feebly developed; the caudal fibres are not only powerfully developed, but are markedly oblique in direction. They often form a well-marked "tail" which lies ventral to the sternum (Figs. 4, 5).

B. *Sphincter colli profundus and sphincter transitus.*[1] In some mammals two sphincter sheets are present in the ventral region of the neck. In the dog (Huber) these are the sph. colli profundus and sph. colli superficialis. The latter

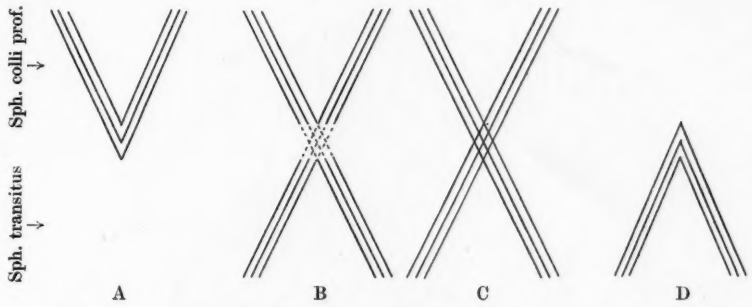

Fig. 1. A line schema to illustrate the transition from the sph. colli profundus to the trachelo-platysma. A. In lower mammals and lesser primates is seen the simple type of sph. colli profundus. B. In rodents the sph. colli profundus has crossed the mid-ventral line to form a sph. transitus. C. In marsupials there is a well-marked break in the continuity of the two sphincters. D. In monotremes and anthropoids the sph. colli profundus disappears leaving the sph. transitus as the trachelo-platysma.

is a delicate and feeble sheet. It may be a Rest or it may be a detached sph. transitus; at present its matrix and its comparative morphology are undetermined. It has never been suggested as the matrix of any other mammalian muscle.

In rodents and marsupials, also, two sphincters are present (Figs. 1 B, C). Here the outer sheet is not the sph. colli superficialis; it is formed by an extension of the sph. colli profundus. The fibres of the muscle no longer merely interlace in the middle line with those of the opposite side; after interlacing they spread across the front of the neck and chest to end upon the arm, shoulder and noto-platysma of the opposite side. There can, thus, be formed an extensive secondary sphincter sheet which lies superficial to the sph. colli profundus. This has been called the sph. transitus. As the relations of the sph.

[1] This term has been coined to avoid using the term sph. colli superficialis falsus, which lacks definition and is both clumsy and misleading. For its definition see text.

transitus are different in rodents and marsupials they will be described separately.

Rodents. The sph. transitus is frequently, though not invariably, present in these mammals; its development is variable. The most complete development is that described by Meinertz in *Cavia cobaya* (1932), where practically the whole of the sph. colli profundus fibres take part in its formation. Here there is no special extension of the caudal fibres.

In the rabbit, on the other hand, the development of the sph. transitus is so feeble that the sph. colli profundus, in this respect, differs little from that seen in other mammalia. Between these extremes many stages have been described by Schreiber (1929). One variety is of particular interest. It is

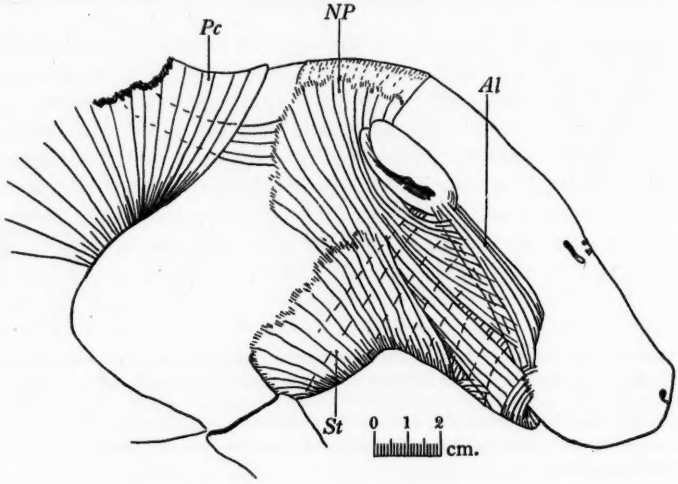

Fig. 2. A dioptogram of the right side of the head and neck of *Hydromys chrysogaster* to show the sph. transitus. The rostral fibres are very feebly developed, but the caudal fibres form a powerful sheet which invests the shoulder and side of the neck and partially covers the noto-platysma. The deeply situated sph. colli profundus is indicated by interrupted lines. (Z.B. 17.)

described by Schreiber in *Castor canadensis*, but is seen more simply displayed in *Hydromys chrysogaster*. In both there is a special extension of the caudal fibres.

Hydromys (Figs. 1 B, 2, 3). The sph. colli profundus is well developed and needs no description. A well-developed sph. transitus is present; rostrally its fibres are so few, delicate and short that they may easily be overlooked; the caudal fibres, however, are very numerous, strong and lengthy; they form a powerful sphincter sheet. Their interlacement in the mid-ventral line extends considerably caudal to the rostral end of the sternum (Fig. 3). The fibres partially cover the anterior chest wall and encircle the arm, investing its medial as well as its lateral aspect, but not reaching the antecubital fossa.

They also clothe the shoulder and, more rostrally, lie superficial to the M. cervicalis transversus and the noto-platysma (Fig. 2). The ventral view of the sph. transitus strikingly resembles that seen in the marsupials; laterally this resemblance is lacking as the muscle passes superficial to, instead of being in the same plane as, the noto-platysma. The fibres of the sph. transitus can readily be traced; they are continuous with those of the sph. colli profundus of the opposite side.

Marsupials. In these animals the sph. transitus lies in the same plane as, is inseparable from and appears to be an expansion of, the noto-platysma; some of the fibres, however, are continuous with those of the sph. colli profundus.

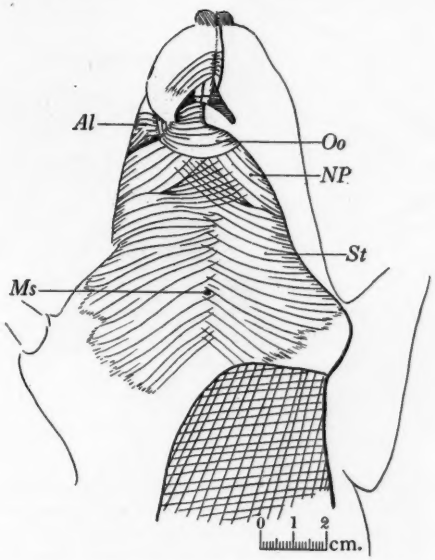

Fig. 3. A dioptogram of the ventral aspect of the head and neck of *Hydromys chrysogaster*. The sph. transitus extends considerably caudal to the rostral end of the sternum; it clothes the front of the chest and arm, but does not reach the antecubital fossa (cf. Fig. 4). (Z.B. 17.)

Some attributes of the sheet, therefore, are distinctive of trachelo-platysma, others distinctive of a sphincter transitus; it would appear to be a stage in transition between the two. As far as could be judged, not more than 50 % of the fibres of the sph. transitus are continuous with those of the sph. colli profundus. The remainder of the fibres of both muscles remain discrete. The relationship between the sphincters is of two kinds: The first, and most usual, is that the two muscles are so closely united in the mid-ventral plane as to be inseparable. This fusion is not a linear one, but band-like in character and is due partly to a binding by fibrous tissue, and partly to an irregular extra-medial exchange of fibres between the two sheets. If the sheets be removed

in toto, carefully cleaned and mounted in Canada balsam between sheets of glass, it will be seen that the fibres of the sph. transitus run in the same direction as those of the sph. colli profundus, and that, at times, the fibres of the two sphincters are continuous. This is well seen in *Trichosurus vulpecula* (Lightoller, 1939).

In the second arrangement, which is less common, the two sphincters can be separated as far as the mid-ventral line. Here they are bound to each other by the interchange of a number of fibres. The best example of this type, so

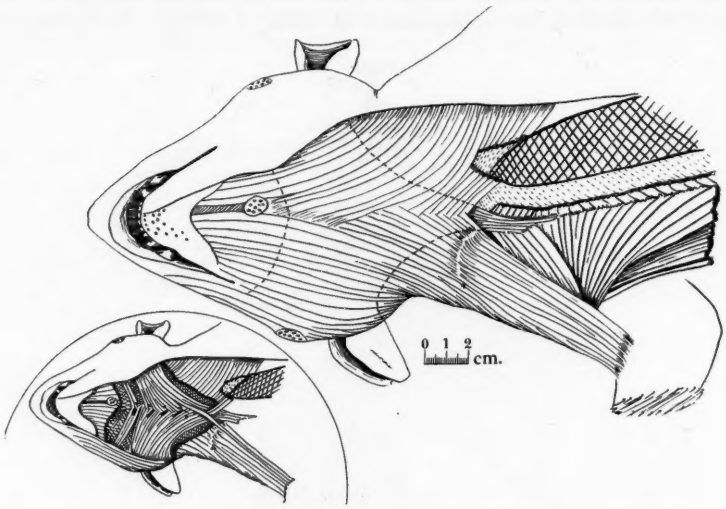

Fig. 4. A dioptogram of the ventral aspect of the head and neck of *Sarcophilus harrisi*. The "tail" of the sph. colli profundus is clearly seen. The decussation of the sph. transitus in the mid-ventral line does not extend caudal to the rostral end of the sternum; its fibres clothe the front of the chest and the arm and extend beyond the antecubital fossa to reach the forearm. The sph. transitus lies in the same plane as the noto-platysma and, superficially, is indistinguishable from a trachelo-platysma. *Inset*. The noto-platysma and the sph. transitus have been partially removed to display the sph. colli profundus. Caudally, some of its fibres are shown crossing the middle line to join the sph. transitus. The other fibres of the sph. colli profundus which crossed the middle line are shown cut across; it is only a percentage of the fibres of the two sphincters which are continuous one with the other. (Z.M. 20.)

far seen, was in *Sarcophilus* (Figs. 4, 5). In the inset (Fig. 4) a large portion of the sph. transitus and noto-platysma has been removed to reveal the sph. colli profundus. To do this numerous fibres were cut which passed between the sph. colli profundus and the sph. transitus; these are indicated. Fig. 5 is a photograph (unretouched) of an actual dissection. Here portions of the sph. transitus and noto-platysma have been freed from the sph. colli profundus and pinned to the opposite side of the neck. The interchange of fibres between the sph. colli profundus and the sph. transitus is revealed. In marsupials the sph.

transitus (trachelo-platysma) covers a portion of the chest wall, clothes the arm medially and laterally and crosses the antecubital fossa to gain insertion into, or become continuous with, the fascia of the forearm. Considered as a primitive trachelo-platysma it fulfils the conditions that were anticipated when the trachelo-platysma of the Cercopithecidae, Anthropoids and Man were investigated (Lightoller, 1928). Four stages in the phylogenetic development of the trachelo-platysma can be recognized: (1) its fibres interlace in the mid-ventral line caudal to the hyoid; (2) its fibres interlace in the mid-ventral line between the hyoid and the chin; (3) some of its fibres are inserted into the

Fig. 5. Photograph (unretouched) of an actual dissection of the neck of *Sarcophilus* to display the interchange of fibres between the sph. colli profundus and the sph. transitus. Portion of the noto-platysma and the sph. transitus of the right side has been reflected as a sheet, and pinned to the left side of the neck.

lower lip, partially replacing the noto-platysma; (4) its fibres are inserted into the modiolar area as well as the lower lip and entirely replace the noto-platysma. Overlapping of these stages is met with.

C. *Sphincter transitus* (*trachelo-platysma*). Here the sph. transitus persists, but its matrix—the sph. colli profundus—is not present in the adult. Work, so far, has not been done to ascertain the conditions present in the embryo.

The only order of mammals in which the whole of the sph. transitus persists without its matrix—the sph. colli profundus—is that of the monotremes. Here the sph. transitus is exceedingly well developed, and not dissimilar in

appearance to that of the *Cavia cobaya* figured by Meinertz. This sheet has hitherto been regarded as a sph. colli superficialis.

More usually only the caudal fibres of the sph. transitus persist. This is the condition which forms the trachelo-platysma in all the higher primates and in Man.

SUMMARY

An attempt has been made to show that the trachelo-platysma of primates may have been derived from the sph. colli profundus of lower mammals. In most mammals the sph. colli profundus consists of a sheet of muscle which covers the ventral and lateral aspects of the neck and the side of the face. It passes deep to the noto-platysma. In rodents, however, the fibres of the sph. colli profundus cross the mid-ventral line to form a second powerful sphincter—the sph. transitus. It covers a portion of the front of the chest, clothes the medial and lateral aspects of the arm and invests the side of the neck and face. It passes superficial to the sph. colli profundus and to the noto-platysma. In certain rodents the rostral fibres of the sph. transitus are very feeble, but the caudal fibres are greatly developed. In marsupials it is the caudal fibres of the sph. transitus that persist. These lie in the same plane as the noto-platysma and are intimately associated with it; they are, however, only partially dissociated from their underlying matrix—the sph. colli profundus. They represent a stage of transition between the sph. transitus of rodents and the trachelo-platysma of primates. In primates the sph. colli profundus is no longer seen and the well-developed caudal fibres of the sph. transitus persist as the trachelo-platysma.

In conclusion I wish to thank Prof. A. N. Burkitt for his continued assistance and the Department of Anatomy, University of Sydney, for granting facilities and material for this work.

ABBREVIATIONS

Figures accompanied by a scale are dioptograms. *Al* = M. auriculo-labialis; *Ms* = rostral end of the sternum; *Oo* = M. orbicularis oris; *NP* = noto-platysma; *Pc* = panniculus carnosus; *St* = sphincter transitus.

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THE COMPARATIVE MORPHOLOGY OF THE M. CANINUS

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The M. caninus was thought by Ruge to be a derivative of the M. orbicularis oris and an adaptation peculiar to primates; he gave to the muscle complex the name M. canino-orbicularis. In the order primates this seemed an apt term for an apparently self-evident truth—the muscles were inseparable.

Theoretically there are objections to both these assumptions, objections weighty enough to require their investigation. As a result, it will appear doubtful whether the M. caninus can be considered either as a muscle peculiar to the primates or as a derivative of the M. orbicularis oris. Adaptations of muscles occur often, and these, at times, are difficult to homologize, e.g. the P.p. orbitalis and jugalis M. maxillo-naso-labialis: new muscles with special functions also occur which may seem, in the present state of our knowledge, impossible to homologize. But these latter are, usually, small muscles which are confined to one particular animal, or one small group of animals. The fact that the M. caninus is found throughout the primates suggests that it is an adaptation of some muscle matrix present in the subprimates rather than a new specialization. Its origin from the canine fossa precludes the M. orbicularis oris as its matrix; the latter is essentially a superficial muscle, whereas the origin of the M. caninus lies deep to morphologically old and long-established nerves and muscles; it has, in fact, the most deeply situated muscle attachment in this area.

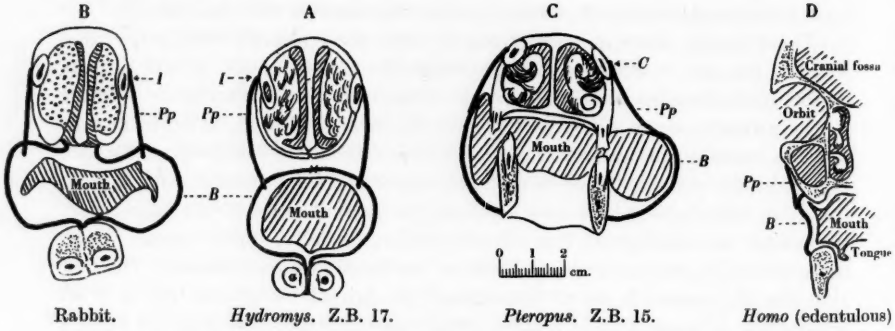
In looking for a matrix for the M. caninus, therefore, it must not have a less deeply situated origin than the other muscles of this area. Further, the origin must be a very large one, extending, possibly, from the bony narial margin to the root of the zygoma. This would be necessary to account for the different types of M. caninus found in primates. In the lemurs and Cercopithecidae the muscle takes origin from the canine jugum where it reaches almost to the bony narial margin; and in the anthropoids from the canine fossa, extending towards the root of the zygoma.

Such a muscle is found quite commonly, though not universally, in the subprimates, viz. the P. profunda M. maxillo-naso-labialis (M. bucco-naso-labialis of Schreiber and Meinertz). Boas and Paulli (1908) describe this muscle in the rabbit as part of the M. buccinator.

P. profunda M. maxillo-naso-labialis. In a previous article (Lightoller, 1934) it was suggested that the muscles taking origin from bone in the orbito-nasal region had, in all probability, a common matrix, and might conveniently

be grouped together under the name M. maxillo-naso-labialis; further, it was shown that this muscle complex consisted of four portions: three of these are superficial, P.p. orbitalis, jugalis and alveolaris, to the deeply situated fourth, P. profunda.[1] It has been suggested that the more superficial are homologous with the capita angulare and infra-orbitale and, possibly, the Mm. labii superioris profundus[2] and nasalis. It is now suggested that the P. profunda is homologous with the Mm. caninus and incisivus superior.

The P. profunda has been described in many rodents, under the name M. bucco-naso-labialis, by Schreiber and Meinertz. In the present series of dissections it has been found in the three rodents dissected, in many marsupials, in three types of the Pteropidae family and in a *Cynopterus sphinx*.



Rabbit. *Hydromys*. Z.B. 17. *Pteropus*. Z.B. 15. *Homo* (edentulous)

Fig. 1. Dioptographic tracings of the rostral surface of oblique sections through the nasal area in four mammals. A. *Hydromys chrysogaster*. The P. profunda takes origin from the incisor jugum and is inserted into the submucosa of the cheek. The caudo-rostral fibres of the M. buccinator are omitted; the dorso-ventral fibres form an almost complete sphincter. B. *Lepus cuniculus*. The arrangement is very similar to the above, but the M. buccinator is attached to bone dorsally as well as ventrally. C. *Pteropus scapulatus*. The P. profunda, in this section, takes origin from the canine jugum and ends superficial to, or interlaced with, the fibres of the M. buccinator. D. *Homo sapiens*. This section was viewed obliquely. The P. profunda (M. caninus) ends similarly to *Pteropus*; it takes origin from the canine fossa. Note that, in all sections, the P. profunda supports the vestibule of the cheek.

In many mammals the muscle, apparently, is not present, e.g. dog and cat (Huber), horse and cow (Sisson & Grossman).

Rodents. In both *Hydromys* and *Notomys* the P. profunda takes origin from the large semicircular incisor jugum. In the former it extends from the rostral end of the jugum to the pre-orbital fibres of the M. masseter (Fig. 2); in the latter it does not extend so far caudally. In both the general direction of the fibres is ventral; but, as the sheet is fan-shaped, the ends have rostral and caudal inclinations. The caudal fibres end by interlacing with those of the M. buccinator; but a large number of the rostral fibres lie rostral to this

[1] This lies deep not only to the other muscles in this region but also to the N. infra-orbitalis.
[2] This has been described and figured in *Homo* (Lightoller, 1928).

muscle, and end in the submucosa of the upper lip (Fig. 2). In both these rodents the dorso-ventral fibres of the M. buccinator are attached ventrally to the dorso-medial edge of the asymphysial mandible; dorsally they are not attached to the maxilla, but end, ventral to the palate, in a median raphe (Fig. 1 A).

Lepus cuniculus. The muscle here is similar to that just described, but complicated by the interlacement, dorsally, of the fibres of the M. naso-labialis (naso-labialis superior of Meinertz). The dorso-ventral fibres of the M. buccinator gain attachment to the maxilla (Fig. 1 B).

Marsupials. In marsupials the muscle is very variable. In *Dendrolagus lumholtzii* the muscle is not dissimilar to that described for rodents, but all the fibres interlace with the M. buccinator, none are attached to the mucosa of the lip.

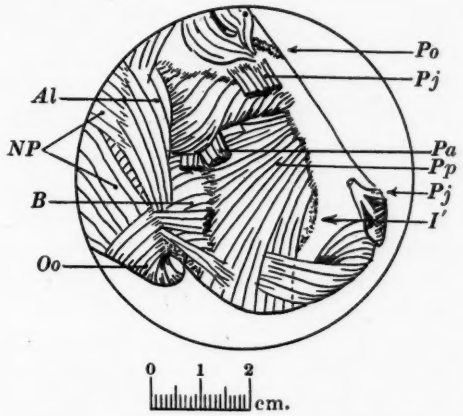


Fig. 2. A dioptogram of the snout of *Hydromys chrysogaster*. The P. profunda takes origin from the semicircular incisor jugum; its caudal fibres interlace with the M. buccinator, its rostral fibres end in the submucosa of the upper lip. (Z.B. 17.)

In *Antechinus swainsonii* and *Perameles nasuta* the muscle is band-like and runs parallel to the alveolar margin. It takes origin from this margin, and its rostro-caudally directed fibres end at the angle of the mouth by interlacing with those of the M. buccinator. The orbicularis oris fibres are feebly developed in the upper lip.

In the koala only the rostral, in *Hypsiprymnodon* only the caudal, fibres are represented; in both, however, the fibres interlace with those of the Mm. buccinator and orbicularis oris.

In *Myrmecobius*, *Dasyurus maculatus* and *Trichosurus vulpecula* the muscle takes origin from the alveolar margin; thence it passes, in a dorso-ventral direction, between the mucosa and the M. orbicularis oris; some fibres end in the former and others interlace with the latter.

In *Sarcophilus harrisi* is seen a stage which is intermediate between that of *Dendrolagus* and *Dasyurus*. Here the muscle consists of two layers; the deep takes origin from the alveolar margin, the more superficial from the bone deep to the tendons of the P. jugalis. Both sets of fibres fuse towards their insertion, which is similar to that of *Dasyurus*.

In *Thalacomys lagotis* the muscle is highly specialized.

Chiroptera. Three varieties of the fruit-eating bat, *Pteropus*, were dissected and the P. profunda is similar in size and form in each. It takes origin, anterior to the canine jugum, from the premaxilla close to the bony narial margin; also from a slightly curved line which extends from here as far caudally as the M. palpebralis medialis.[1] The most caudal fibres may lie dorsal or ventral to

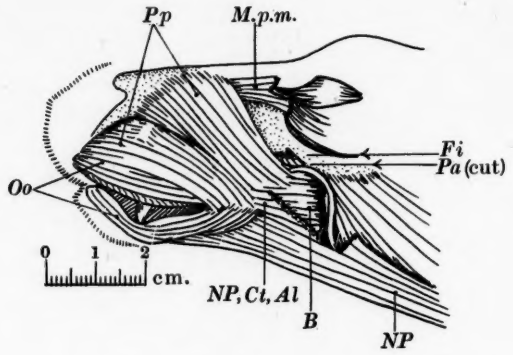


Fig. 3. A dioptogram of the snout of *Pteropus scapulatus*. The P. profunda is large. Its origin extends from an area rostral to the canine jugum to an area dorsal, sometimes ventral, to the M. palpebralis medialis. It passes superficial to the Mm. orbicularis oris and buccinator and interlaces with both these muscles. (Z.B. 15.)

this; in either case they approach the root of the zygoma (Fig. 3). The P. profunda sheet tends to divide into rostral and caudal portions (Fig. 3). From this wide area of origin the fibres pass ventro-caudally to end by interlacing with, or to lie superficial to, the Mm. orbicularis oris and buccinator (Figs. 1 C and 3). In *Pteropus* the M. buccinator does not consist of dorso-ventral and caudo-rostral sheets; its fibres run obliquely and are attached, directly or indirectly, to the lateral surfaces of the maxilla and mandible (Fig. 1 C).

In *Cynopterus sphinx tithaechailus* only the caudal fibres are present, but these end by interlacing with those of the Mm. orbicularis oris and buccinator.

Primates. In primates there is not found any large P. profunda such as has been described above; the rostral and caudal portions are represented by two separate deeply situated muscles, viz. the Mm. incisivus superior and caninus.

[1] This term has been used because the attachment of the M. orbicularis oculi to the maxilla is muscular and not ligamentous.

The caudal portion is effectively represented by the M. caninus; the rostral portion, however, is far more variable in its mode of origin.

To cover by a single name these variations, a wider view must be taken of the area from which the M. incisivus superior may take origin. It may be defined as a muscle which takes origin from the neighbourhood of the ventral or lateral margins of the bony anterior nares, becomes closely associated or fuses with the M. orbicularis oris, and ends, when able to be separately traced, in the modiolar region.[1]

As might be expected, in some animals there will be fibres from the ventral as well as fibres from the lateral margin of the bony nares, each of which may join the M. orbicularis oris independently; in other cases it may be difficult to decide whether the muscle should be called a M. caninus or a M. incisivus.

The lemurs (Z 06 and Z 07) (Lightoller, 1934) possess only a M. incisivus superior. A few weak fibres take origin ventral to the bony nares, but a large sheet of fibres takes origin lateral to the fossa.

In the Cercopithecidae (Lightoller, 1928) a similar condition is found, but both groups of muscle fibres are well developed. In neither of these groups is a M. caninus present.[2]

In the gorilla (Ruge, 1887), orang (Lightoller, 1928) and *Homo* are present a M. incisivus superior taking origin from the incisive fossa and a M. caninus from the canine fossa. Both are deeply situated and represent the rostral and caudal portions of the P. profunda. No fibres take origin from the lateral margin of the bony nares.

In primates, therefore, the P. profunda of lower mammals is represented in two ways: the whole of the rostral fibres may remain with a complete absence of the caudal ones; or, the infra-narial rostral fibres and the whole of the caudal fibres may be present.

Function. The complete enumeration of the functions of the P. profunda in any or all of the above-mentioned orders could only be ascertained by a prolonged and careful study of the animals in question. But a simple function seems common to all—even to *Homo*, viz. to support the vestibule of the cheek and upper lip (Fig. 1). In Man, of course, it has been adapted for the production of sound, especially speech.

SUMMARY

A brief account has been given of the P. profunda M. maxillo-naso-labialis in certain selected mammals and in Man. It has been shown that the muscle is frequently, though not invariably, present in the Mammalia; but insufficient material is at present available to determine either the frequency of its

[1] The fibres taking origin lateral to the bony nares are neither true caninus nor true incisivus, but from the condition found in *Pteropus* it seems better to class them with this than to give them a new name.

[2] Those fibres which take origin from the lateral margin of the bony nares have, hitherto, been classed as M. caninus.

occurrence or of its presence or absence in any particular order. The muscle has been shown to support the vestibule of the cheek and upper lip, and the Mm. incisivus superior and caninus of Man have been homologized with it.

In conclusion I wish to thank Prof. Burkitt for his assistance and for his helpful criticism, and the Department of Anatomy, University of Sydney, for the material and facilities wherewith to conduct this investigation.

ABBREVIATIONS

Figures accompanied by a scale are dioptograms. *Al*=M. auriculo-labialis; *B*=M. buccinator; *C*=canine tooth; *Ct*=M. cervicalis transversus; *Fi*=foramen infra-orbitale; *I*=incisor tooth; *I′*=incisor jugum; *M.p.m.*=M. palpebralis medialis; *NP*=noto-platysma; *Oo*=M. orbicularis oris; *Pa*=P. alveolaris; *Pj*=P. jugalis; *Po*=P. orbitalis; *Pp*=P. profunda.

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CEPHALOMELUS: A RARE TUMOUR IN A LAMB

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Cephalomelus may be defined as that type of monster in which an accessory limb (or limbs) is inserted on the head. The condition seems to be sufficiently rare to justify the description of another case. I can trace only three recorded cases in the literature, those of Tiedemann (1831), Geoffroy St Hilaire (1836) and Gosselin (1857). The first two of these cases occurred in ducks and the last in a dog. The abnormality has not been recorded in man.

This specimen was obtained from a cross-bred Kerry Hill-Welsh lamb. Pregnancy and birth were normal. The lamb, a male, showed marked right-sided cerebellar symptoms and signs. There was a large mid-line, bony-hard, lobulated tumour projecting backwards for 1½ in. from the occipital region. The tumour was not fixed to the skin but was firmly adherent to the underlying bone. The animal was bottle-fed and lived for 8 days, eventually dying of lamb dysentery.

A radiograph of the tumour *in situ* is shown in Fig. 1. On dissection the tumour was found to pass through an aperture in the occipital bone and was firmly fixed to the margins by dense fibrous tissue. Inside the cranial cavity it passed above the tentorium cerebelli as far as the mid-brain. The roof of the mid-brain was deficient and the colliculi incompletely developed. The cerebellum was markedly flattened from above downwards and the occipital lobes deeply indented. No difference was noticed between the right and left sides.

The radiograph of the dissected tumour (Fig. 2) shows the distal ends of a right and left femur with well-developed centres of ossification. The cartilage of the femoral epiphyses is continuous with another mass of cartilage and bone which consists of the proximal end of a single tibia and a single fibula; there are two distinct bony epiphyses surmounting the single tibial shaft; there is no joint cavity between the epiphyses of the femora and tibiae. The tumour thus displays the conditions of sympodia in which fusion of the two limbs has occurred in the region of the proximal ends of the tibiae, so that the tumour itself is an example of sympodia of the type sympus apus in which the fused limbs end in a stump.

The limb bones in the tumour show well-marked lines of arrested growth near the ends of the shafts and resemble those described by Harris (1933). These lines correspond with those in the radiograph of the knee joint of the host and indicate that before and after birth the interferences with growth affected the bone of the tumour and the host in the same manner.

Histological examination of a small piece of the bony shaft of the tibia showed no definite cytological anomaly.

The autosite showed no other abnormalities.

Fig. 1. Radiograph of the skull of the lamb with the tumour in the occiput.

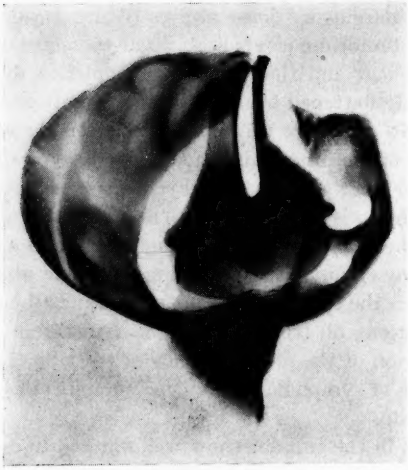

Fig. 2. Radiograph of the tumour displaying the distal ends of the left and right femora with their bony epiphyses above and the single tibia below.

SUMMARY

This monster belongs to the group of cephalomelus. In addition to the rarity of the condition, it shows two interesting features:

(1) The presence of lines of arrested growth in the parasite identical with those in the autosite.

(2) Partial fusion of the lower limbs in the parasite, with absence of the knee joint cavity.

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ANATOMICAL NOTES

NOTES ON THE CONSTRUCTION OF BLOTTING-PAPER MODELS

By R. L. de C. H. SAUNDERS

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Embryological models made of blotting-paper were demonstrated for the first time in 1905 by Mrs S. P. Gage at a meeting of the Association of American Anatomists, a general outline of the method appearing subsequently (1907) in the *Anatomical Record*.

Since then, both Miller (1931, 1932) and Parsons Schaeffer (1911) have pointed out the advantages of this method of making models and have added materially to it. It is an excellent method, and yet many seem content with the Born wax-plate method of reconstruction with its attendant expense and fragile end-product.

The object of this paper is to point out some of the difficulties which may be encountered and how best they may be overcome, as well as to suggest a modification of the existing technique.

1. Blotting-paper

Measurements are rapidly and easily calculated from models built at a magnification of 100 diameters, and it is therefore a very satisfactory magnification to employ. Should the specimen on which the model is based be cut at 10 μ, sheets of 1 mm. thickness are obviously necessary, if uniform magnification of all dimensions is to be obtained.

Thick sheets of blotting-paper are expensive. Fairly uniform sheets of 1 mm. thickness may, however, be prepared quite economically if three sheets of Ford Mill 428, thinly coated with household decorators' paste, are rolled with a photographic roller and allowed to dry. The paste used is absorbed by the blotting-paper and is the same as that which I employ in "piling" the sections. It is described later.

Other magnifications require sheets of varying thicknesses. If sheets suitable to the magnification cannot be obtained, Mrs Gage explains how one may overcome this difficulty by using extra sheets at regular intervals or by omitting a section.

2. Drawing the sections

Using a camera lucida or projection microscope, Mrs Gage apparently made her drawings direct on to blotting-paper.

My own experience has been that besides the blotting-paper tending to tear, its surface does not lend itself to the drawing of minute detail. As Miller pointed out, the drawings should be made on a bond paper and transferred by means of carbon paper. This method has the additional advantage that the drawings may be preserved as a record or may be employed in the construction of other models.

At this stage of construction it should be borne in mind that a great source of error in model-building is the spherical aberration which distorts the image on the drawing surface. Geddes (1911), in his paper on the "Technical Difficulties of Wax-Plate Reconstruction", has pointed out how best this may be avoided.

3. Cutting the sections

Mrs Gage, who was the first to introduce the sewing-machine as a medium for cutting out the sections, later improved on this method by using an electric machine.

Miller found the use of a sewing-machine impracticable in the construction of large

models, owing to the distance between the needle and the supporting arm of the machine being too short. He therefore resorted to the Cutawl, a machine designed for use by commercial artists. This instrument, which is a complete unit in itself, is placed on the blotting-paper and can be moved freely to cut out the desired sections, no matter how large they may be. For general purposes, an ordinary second-hand sewing-machine will be found to be quite satisfactory. A treadle machine with a heavy flywheel should be selected—the heavy flywheel ensuring smooth movement.

The feeding arms at the sides of the needle tend to obscure one's view of the line and one of these should therefore be removed if the section outline is to be rigidly adhered to. This, I found, does not in any way impair the efficiency of the feeding mechanism.

The shuttle also should be removed and the stitch regulator set to give the shortest possible stitch and consequently an almost continuous perforation. Chisel-shaped needles have been recommended, but are not essential.

4. Piling

Various methods have been recommended for fixing the sections together. Finding no kind of paste or glue suitable for fixing the sections together, Mrs Gage employed pins and wire nails. Miller's experience was different, in that he found two pastes—Stickine and his Stickine substitute—which answered the purpose. I have used Henkel's Cold Water Decorators' Paste Powder[1] with satisfactory results. The powder is stirred into cold water, and forms an excellent smooth paste with it.

Paste powder, which is cheap, keeps indefinitely. These facts, coupled with the ease and rapidity with which it may be prepared, render it a more satisfactory mixture than those hitherto suggested.

The necessary dissections are made during the process of piling, and the model, when completed, is allowed to dry. Processes, such as the insertion of reinforcing wire and the removal of bridges, may now be carried out with safety.

Instead of resorting to the usual practice of coating the model, or immersing it in paraffin wax, I coat with a mixture of plastic wood and acetone. The plastic wood is slightly thinned down with acetone and is best applied by the fingers. When dry the model is smoothed with sand-paper.

Dissection may be deferred until this stage has been reached. The dissected parts are strong, and do not tend to stick together as in wax models.

5. Colouring

Wax-impregnated models do not readily take colour. Mrs Gage employed coloured lens paper, moulded over the surface of the model and held in place with a coat of hot paraffin, or a mixture of oil paint and hot paraffin, while Miller has evolved a method of simultaneously assembling and colouring his models, which, in short, depends upon the mixing of colour with his piling paste, or the use of coloured blotting-paper.

Having tried these, I resorted to poster colour, which I found gave satisfactory results. Wax models should be coated with a 5% gelatine size before being coloured with poster colour, and then recoated with size before being varnished.

In order that this rather lengthy process may be obviated the poster colour may be diluted with warm 5% gelatine size and applied immediately after dilution. This coating dries quickly and the model is then ready for varnishing.

On the other hand, models finished with plastic wood take any form of colouring agent, and I have found Chinese lacquer gives an excellent high gloss finish.

These models, which are cheap to build, are generally more satisfactory than wax-impregnated ones. Attractive features are the cleanliness, ease and rapidity with

[1] Available at most household decorators.

which they may be constructed. They are strong and therefore very suitable for teaching purposes.

Since completing these notes, Green's paper (1937) on the "Technique of Plastic Reconstruction" has come to my notice. Although approximating to the Born wax-plate method of reconstruction, I see fit to mention his method here, in that (1) recognizing the expense of the Gage method, he suggests the use of "4-sheet pulp board 44 × 32 in.", impregnated with paraffin wax (melting point about 55° C.) in order to obtain sheets of approximately 1 mm. thickness and at the same time facilitate the process of cutting, and (2) presumably recognizing the fragility of "wax-plate" models he recommends that the finished "wax" model be coated with plastic wood to give it greater rigidity.

Although he does not state the method of cutting these plates, he presumably uses a knife. The use of a sewing-machine is an essential feature of the Gage method, in that it greatly speeds up the whole process of construction.

Plastic wood he finds "useful to fill in the 'steps' in the completed model (if it is desirable to do so)", and from my experience of the substance I should say that it would greatly increase the strength of a "wax" model, as he suggests.

It should be emphasized that I employ no wax whatever, finding that blotting-paper sections piled with Henkel's paste and allowed to dry thoroughly before receiving an application of plastic wood and acetone give a light model of great strength.

Summary

1. The notes, which review the work of others, also deal with the economical preparation of millimetre sheets of blotting-paper, a new piling paste, and a gelatine-poster colour method of colouring wax models.

2. A modification of the Gage technique, which dispenses with the use of paraffin wax, is urged, in that these models, which are simply built of blotting-paper piled with Henkel's paste and coated with a mixture of plastic wood and acetone, take any form of colouring agent, while their lightness and strength render them very suitable for teaching purposes.

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IN MEMORIAM

ROBERT WILLIAM REID

ROBERT WILLIAM REID, Emeritus Professor of Anatomy in the University of Aberdeen, died at Aberdeen on 28 July 1939, in his eighty-ninth year. He was the last of the original members of the Anatomical Society of Great Britain and Ireland which was founded in 1887; he was elected to its first Council and later filled with distinction the office of President.

Professor Reid was born in the manse of Auchindoir, Aberdeenshire, in 1851, being the third son of the Reverend William Reid, minister of the parish. He studied at Aberdeen University, graduating M.B., C.M., in 1872 and M.D. three years later; in 1881 he became a Fellow of the Royal College of Surgeons of England. He began his career as an anatomist in his Alma Mater by acting as assistant for one year to the late Professor Sir John Struthers. Thereafter, at the early age of twenty-two, he was appointed Demonstrator of Anatomy in the Medical School of St Thomas's Hospital, London, becoming Lecturer in Anatomy in 1877. At that time there was only one professional anatomist in London—the Professor at University College. Reid not only reorganized the anatomy department at St Thomas's but found time for research, and in the *Lancet* for 27 September 1884, published "Observations on the relation of the principal fissures and convolutions of the cerebrum to the outer surface of the scalp"; brain surgery was in its infancy and this pioneer research supplied surgeons with a simple and reliable guide. Another important paper on topographical anatomy was published by him in the *Journal of Anatomy* on the relationship of nerve roots to vertebral markings.

In 1889 he was appointed to the Regius Chair of Anatomy in his own University and held that Chair for thirty-six years, retiring in 1925. Here his work as a teacher, investigator and organizer was marked with that thoroughness and detailed tenacity of purpose so characteristic of the man. Never did he spare himself in his efforts on behalf of his department. In the closing years of the nineteenth century Anatomy was still accepted as a "descriptive science"—minute, elaborate, and accurate description of parts without regard to significance or function. A department of Anatomy had little equipment beyond the cadaver. Professor Reid conceived of Anatomy as the necessary foundation of Physiology and the basis necessary to all medical investigation. He planned and obtained new rooms for practical work and for research, and refurnished the museum. Under his care the teaching and the equipment of the department was greatly extended and a Lectureship in Embryology instituted. One of the first anatomists to grasp the importance of X-ray examination in relation to his subject, he installed in 1902 an X-ray apparatus—in those early days naturally of a somewhat primitive type.

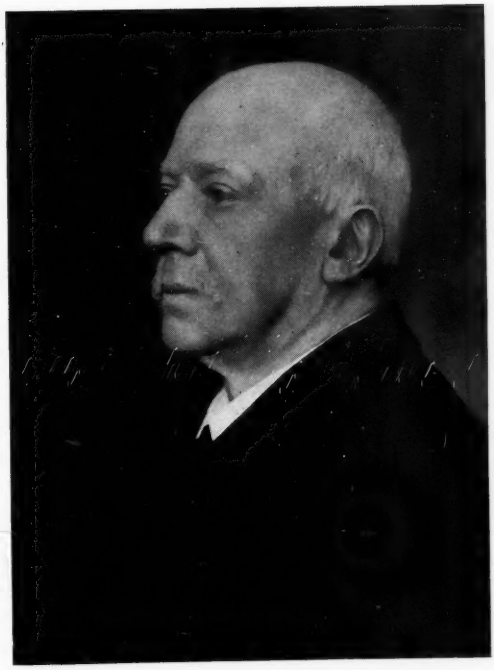
After his retirement he retained his interest in the department and expressed his appreciation of the development in radiology as the result of which in 1929 Lord Beaverbrook's gift enabled a fully equipped radiological outfit to be added to the department. Further, he welcomed the good fortune of the recent arrangement whereby histology, neurology, and experimental research have returned to the department of Anatomy.

As a Fellow of the Royal Anthropological Institute of Great Britain and Ireland Professor Reid was particularly interested in Anthropology, and in 1896 he instituted an anthropometric laboratory where the physical characteristics of students were (and still continue to be) recorded. Three important papers based on the data from these records he published in collaboration with J. H. Mulligan, M.B., in the *Journal of the Anthropological Institute* for 1923 and 1924; these papers dealt with the relation of cranial capacity to intelligence, the relation between stature, head breadth, and head length of natives of the north-east of Scotland, and an anthropometric comparison between natives of the north-east of Scotland and those of Norway and Sweden. One of the most interesting activities in connexion with anthropological study in the University was the formation of the "Aberdeen University Anatomical and Anthropological Society". This society, conceived and started by students in 1899, has continued to flourish to the present day.

One of Professor Reid's most outstanding achievements was the initiation of the University Anthropological Museum. He acted as honorary curator of this museum from its inception in 1907 until 1938, and in its development gave abundant evidence of his pertinacity and capacity for sustained exertion. The University already possessed several valuable archaeological and ethnological collections and these he brought together and rearranged. Thus was formed a nucleus from which has developed a noteworthy museum by the addition of specimens given by many graduates and friends of the University. A notable benefactor to the museum was the late Sir William Macgregor, a distinguished medical graduate of the University, a successful colonial administrator and anthropologist in the widest sense of the term. To him the museum owes its magnificent collections from Fiji, New Guinea, Lagos and Newfoundland. In 1912 Professor Reid prepared a catalogue of the Macgregor Collection and in the same year completed and published an *Illustrated Catalogue of the Anthropological Museum*. In 1928 he published, in collaboration with G. M. Morant, D.Sc., in *Biometrika* "A study of the Scottish short crania". In appreciation of his outstanding work in the development of Anthropology in the University of Aberdeen the Reid Lectureship in Anthropology has been instituted.

During his thirty-six years as Professor of Anatomy, and for thirteen years as Emeritus Professor, in the fulfilment of the duties of his Chair, as Dean of the Faculty of Medicine, as a Member of the University Court and representative of the University on the General Medical Council, Professor Reid rendered conspicuous service to his University.

A. L.



ROBERT WILLIAM REID

UNIV. OF MICH.

REVIEWS

Anatomy of the Sheep's Brain. By E. A. Brigg, D.Sc. (Sydney: Angus and Robertson, Ltd.), 1939. Pp. xiii + 50, 8 Plates and 4 Text-figs. Price 6*s*.

This book, primarily intended for students who are entering upon a junior course of Practical Zoology, provides a concise and easily understood account of the gross features of the sheep's brain and of a method of dissecting it. The plates illustrating it are clearly drawn and add materially to the value of the work, which altogether seems to fulfil admirably the purpose for which it was written.

Human Histology, a guide for medical students. By E. R. A. Cooper. (London: H. K. Lewis and Co. Ltd.), 1939. Pp. xiii + 423, 237 figs. Price 16*s*. net.

This text-book deals essentially with the microscopical structure of post-mortem human tissues and organs, including the central nervous system. The photomicrographs, with which it is illustrated, will be found useful as a basis for comparing the post-mortem appearance of normal human material with the abnormal examined in routine pathology.

Whilst it is desirable that medical students should examine the microscopical structure of normal human material wherever possible, the correlation of histological and cytological structure with physiological function should be given at least equal consideration in a course of histology. In this text-book the references to function are quite inadequate and in some cases they are inaccurate and misleading. In fact, the modern literature of histology has been largely omitted and no bibliography is given for the intelligent student to extend his reading.

Statements such as "the eosinophil cells of the pars anterior are known to produce a 'growth' hormone" would be more convincing to the average student if accompanied by the evidence, experimental and otherwise, on which this belief is based. Similarly, the assertion that lymphocytes are deposited from the blood-stream into the sinusoids of the bone marrow, where they become haemoblasts, should be supported by experimental evidence with some reference to the fact that haematologists still fail to agree on this subject. In addition, the student will find some difficulty with terminology, as for instance in the statement that the endocardium "is continuous with the endothelium of the blood-vessels and consists of pavement epithelium supported by a delicate stroma comprising the subendothelial tissue". K. C. R.

Principles of Development. By Paul Weiss. New York: Henry Holt and Co., 257 Fourth Avenue, New York. Pp. v + 601. Price $5.00.

During the last twenty years or so anatomists have been made acquainted with and have admired the brilliant researches of Dr Weiss, as they have been reported from time to time in Roux's Archives and in American Journals. They represent a pioneer exploration of several fields of experimental anatomy, and they have involved the application of experimental methods notable for their ingenuity. The appearance of a book by the author dealing comprehensively with the principles of development is now extremely welcome. In this book, which is exceptionally well produced and well illustrated, the various factors which initiate and determine structural organization are considered and discussed with a wealth of illustrative example. In the first

part, the phenomena of development are reviewed—the essential features of the growth of tissues and organs, the physico-chemical changes which underly them, problems of relative growth, cellular differentiation, and so forth. In the second part of the book, embryological methods are discussed, with special reference to the technique now employed by the experimental embryologist. In the third part, concepts of determination in development, field-patterns, metabolic gradients, reintegration, etc. are discussed in considerable detail. Lastly, Part 4 deals with neurogenesis and the organization of the nervous system. It will be apparent that the scope of the book is wide. It is written in an attractive style and is fully annotated. The fact that it is the work of so distinguished an anatomist as Paul Weiss will alone persuade anatomists in general to add it to their essential library equipment.

The Anatomy of the Human Skeleton. By J. Ernest Frazer, D.Sc., F.R.C.S. 4th edition. (London: J. and A. Churchill Ltd.) 1940. Pp. viii+300, with 219 illustrations, many in colour. Price 30*s.* net.

We welcome the appearance of another edition of this deservedly popular book and congratulate its author on its continued success. While there are those who may suggest that in its insistence on unessential detail it runs counter to recent trends of anatomical teaching, few will underestimate its value where it inculcates the importance of accurate observation. The introduction of the Birmingham terminology in the text brings this work now into line with all the other standard text-books of human anatomy published in this country.

OBSERVATIONS ON THE NEUROHISTOLOGICAL BASIS OF CUTANEOUS PAIN

By the late H. H. WOOLLARD, GRAHAM WEDDELL and J. A. HARPMAN

From the Department of Anatomy, University College, London

The hypothesis that characteristic morphological nerve endings subserve specific modalities of sensation is widely accepted. Conclusive histological evidence in support of this theory has not, however, been forthcoming. In this paper, the relation of cutaneous pain to the doctrine of specific nervous activity is discussed in the light of new physiological and histological observations.

PHYSIOLOGICAL INVESTIGATIONS

Experiments were carried out on six human subjects. Pain was aroused by the introduction of either glass or steel needles into the skin. Deformation was reduced to a minimum by the use of fine, sharp needles inserted at right angles to the surface under vision through a dissecting microscope. Touch and cold spots were identified by methods previously described (Woollard, 1935). Warmth spots were mapped out by means of electrically heated platinum loops 1 mm. in diameter.

Penetration experiments

The introduction of fine, sharp needles into the skin of the forearm may arouse either no sensation whatever, or sensations of touch, pain, pressure, cold or warmth. Insertion of the needle into previously identified warmth or cold spots frequently arouses the corresponding sensations.

When a fine, sharp needle is used, no pain is felt until penetration occurs. Two types of pain can be aroused, the first being abrupt in onset, hurting little, and lasting for a period corresponding to that during which the stimulus is being applied; the second is delayed in onset, rises gradually in intensity, gives the impression of a small stinging area and disappears slowly. The phase of maximum intensity may occur as much as 2 sec. after the application of the stimulus, even if this be withdrawn immediately

Both types of cutaneous pain are obtained with equal frequency in the forearm; they may occur independently or successively during the course of a single penetration. When a fine, sharp needle penetrates rapidly and deeply, the delay in onset of the second pain is usually greater in the distal than in the proximal parts of the extremities. Sometimes, however, throughout the extremity the interval between the two sensations of pain may be so brief that

a single prolonged phasic sensation is experienced. Two pain sensations, separated by a distinct interval, as well as phasic sensations, can be aroused from the skin of the face, forehead, neck, upper part of the chest and shoulders. Two distinct types of pain cannot be provoked from the skin of the back or abdomen, where successful stimulation leads only to phasic sensations. The distance from the skin surface at which the fundamental modalities of cutaneous sensation can be aroused was determined by the following method. The forearm, wrist and hand were rigidly held in a plaster cast, which left a portion of the volar aspect of the forearm exposed. A very fine needle was clamped vertically over the exposed area in a rack and pinion mount. The needle was lowered into the skin under vision through a dissecting microscope and the depths of penetration read off directly. The measurements were confirmed by painting the protruding shaft of the needle with Indian ink, after particular sensations had been aroused, and determining the length of needle free from ink after withdrawal.

Table I. *Levels at which various sensations are aroused in the skin of the forearm*

Sensation aroused	Depth of penetration
Pressure	2·0–2·5 mm.
Warmth	1·75–2·5 mm.
Touch	Surface contact–2·0 m.
Cold	1·0–1·5 mm.
Second or deep pain	1·0 mm. (average)
First or superficial pain	0·25–0·5 mm.

When the surface of the skin is stimulated by rapid, brief application of bristles or glass beads of not more than 0·5 mm. in diameter, touch, accompanied by both pain sensations, is aroused. The sensation can be described as one of pricking touch. Itching often follows the application of such stimuli.

Observations on cutaneous sensation during and after removal of the epidermis

An area of skin on the forearm was scraped with a scalpel so as to remove as much of the epidermis as possible without drawing blood. There was no change in the frequency or distribution of either pain or warmth spots. Cold spots, however, were more numerous. The thresholds for all three sensations were reduced. Pain resulted from slight friction or contact.

No pain was aroused during removal with a razor of as much epidermis as possible without causing bleeding. The area soon became hyperalgesic, and accurate measurements of levels at which pain was provoked were difficult to obtain. The hyperalgesia was allayed by covering the denuded surface with lint smeared thickly with lanoline for a period of 6 hr. Penetration experiments using needles were then carried out. The relation of the point of the needle to dermal capillary loops was observed through a dissecting microscope. No pain

was aroused until the needle reached the level of the capillary bed. At this subepidermal level, the pain provoked was of the type aroused in normal areas by needles penetrating to depths of around 1 mm., and was of the usual intensity. The more superficial variety of pain could also be aroused at depths of around 0·5 mm. from the lanoline-covered normal margin surrounding the denuded area. Hyperaesthesia to cold was abolished by the ointment.

Relation of the subepidermal pain mechanism to capillaries

Dermal capillary loops were rendered visible by the application of cedar-wood oil to the skin overlying the nail bed. Pain was experienced when the point of the needle lay at the level of a capillary loop. The sensation aroused was similar to the delayed, more intense type of cutaneous pain.

Text-fig. 1.

Text-fig. 2.

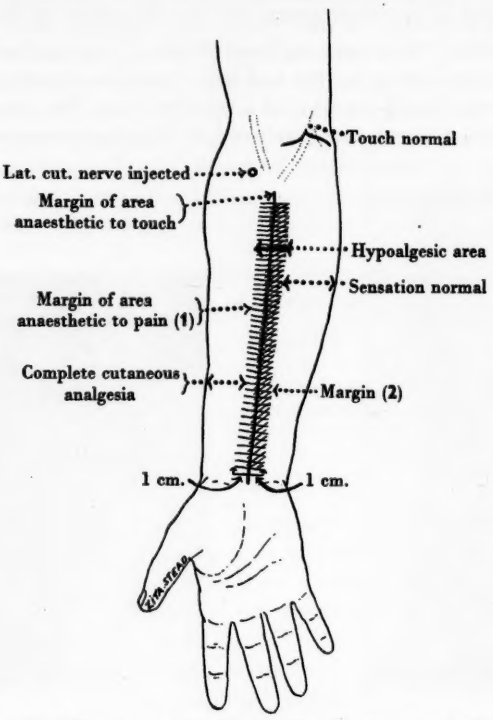
Text-fig. 1. Photograph of a forearm in which complete anaesthesia of the lateral and media cutaneous nerves was obtained on successive days. There is no detectable overlap of touch (centre line). Pain overlaps for a distance of approximately 1 cm. on either side of this line.

Text-fig. 2. Photograph of the volar aspect of a forearm in which complete anaesthesia of the lateral cutaneous nerve had been obtained. The margin of the area insensitive to pain is represented by a broken line, the margin of the area anaesthetic to touch by a continuous line. The distance between the two margins is approximately 1 cm.

Sensory dissociation experiments

Cutaneous nerves of the forearm and digital nerves in the upper extremity were anaesthetized by infiltration, around the trunks, of 2–4 c.c. of 4 % novocain containing 1 in 10,000 to 1 in 20,000 adrenalin. The nerves were located by faradic stimulation (Thompson & Inman, 1928).

When anaesthesia of either the medial or lateral cutaneous nerve of the forearm is complete, the margins of the respective areas anaesthetic to touch on the volar aspect of the limb coincide within the limits of experimental error. The margins of the areas anaesthetic to pain, on the other hand, fall short of those anaesthetic to touch, due presumably to overlap of fibres from the adjacent nerve (Text-figs. 1, 2). No sensation other than pain can be aroused



Text-fig. 3. Volar aspect of forearm. Areas in which ≡ = superficial pain, ▨ = deep pain, can be aroused from the hypoalgesic area. (1) The number of spots from which pain can be aroused is least near this margin. The number increases gradually through the hypoalgesic area until margin (2) is reached where the frequency is normal.

from the area of overlap. The distance between the margins of the areas anaesthetic to pain and to touch is approximately 1 cm. on the forearm, somewhat less than 1 cm. on the thenar eminence, and undetectable on the fingers. When anaesthesia is incomplete, the area anaesthetic to pain may be larger or smaller than that insensitive to touch.

When anaesthesia of the medial or lateral cutaneous nerves of the forearm is complete, the number of spots from which pain can be aroused is below normal on either side of the margin of the area anaesthetic to touch for a

distance of 1 cm. In the area from which pain only can be provoked, the superficial type alone is obtainable to within 3 mm. of the margin of the area insensitive to touch. From the remainder of the hypoalgesic area, both varieties of pain can be aroused (Text-fig. 3).

An ulcer was made on the volar aspect of the forearm with solid carbon dioxide. Eight days later, the ulcer was 5 mm. in diameter and the base extended into the dermis. The margin of the ulcer was hyperalgesic. In an area of skin of 4 sq. mm. situated immediately distal to the ulcer, pain only could be aroused. In the remainder of the surrounding skin, touch and temperature sensations could also be aroused.

Sensory localization experiments

Needles and electrodes supplied with induction shocks (Lewis, 1936, 1937*b*) were used. The minimum distance at which two successive stimuli provoking pain can be recognized as having been applied at different sites is about 1 cm. on the forearm, about ½ cm. on the palm of the hand, and too small to be accurately determined on the pad of the finger. Two stimuli arousing pain of equal intensity applied simultaneously are perceived individually when the distance between them is not less than 1 cm. across the forearm, 1·2 cm. along the length of the forearm, 3–7 mm. on the palm of the hand, and 1 mm. on the pad of the finger. When the distance between such stimuli is less than 1 cm. on the forearm, pain appears to emanate from a single point, but is of greater intensity than the pain provoked by a single such stimulus. The localization of both the superficial and deep varieties of cutaneous pain is of the same order of accuracy.

Two-point discrimination of tactile stimuli averages 2 mm. on the volar aspect of the forearm.

Observations in a case of antero-lateral chordotomy

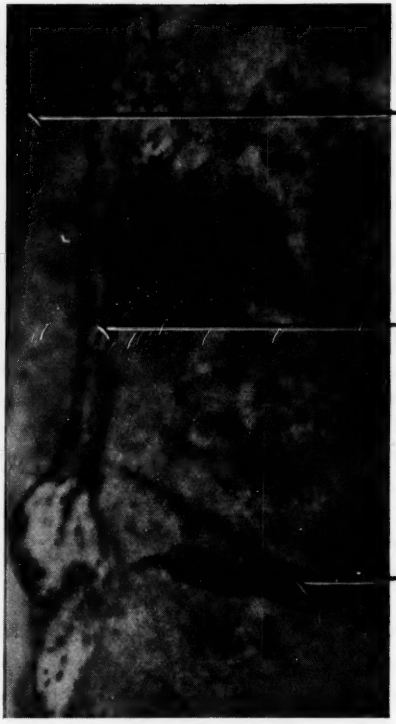
Superficial and deep cutaneous pain, cold, and warmth could not be aroused from the affected regions of the body. The sense of touch was apparently unimpaired. Itching was not experienced in the analgesic area subsequent to operation.

HISTOLOGICAL INVESTIGATIONS

The innervation of the skin and blood vessels of the ear of the rabbit

Staining technique. Clark *et al.* (1934) stained nerve fibres supplying capillaries in the ear of the rabbit *in vivo* by injecting small volumes of methylene-blue solutions into local blood vessels. Their observations suggested the possibility of experimenting upon cutaneous nerves and nerve endings in the surviving animal. The subcutaneous and intradermal injection of solutions of methylene blue in normal saline into the ear of the rabbit was found to stain

nervous tissue selectively (Text-fig. 4). Concentrations of 0·005–0·5 % methylene blue were used. The best average results were obtained by using a concentration of 0·01 % methylene blue in normal saline solution. Precautions to be observed in preparing the solutions have already been described (Weddell *et al.* 1940). The volume of solution injected was between 1 and 1½ c.c. at each puncture, the number of injections depending upon the area it was desired to



Text-fig. 4. Photomicrograph showing nerves stained *in vivo*. Rabbit ear. ×550.

investigate. Observations were made after shaving the ear and rendering the surface layers of the skin transparent by the application of olive oil or liquid paraffin. Nerves and nerve endings did not stain satisfactorily when the local vessels remained constricted after the injection. Good results, however, were obtained when the dye was introduced into ears in which the vessels were at first constricted, but dilated by warmth 10 min. after the injection. Good results were also obtained when the dye was introduced into ears the vessels of which were dilated, provided the circulation was arrested for 10 min. subsequent to injection. Particularly good results were obtained in animals

previously subjected to cervical sympathetic neurectomy. Permanent preparations were made by removing pieces of skin in which the nerves and their endings were stained, and fixing and clearing them in the usual way.

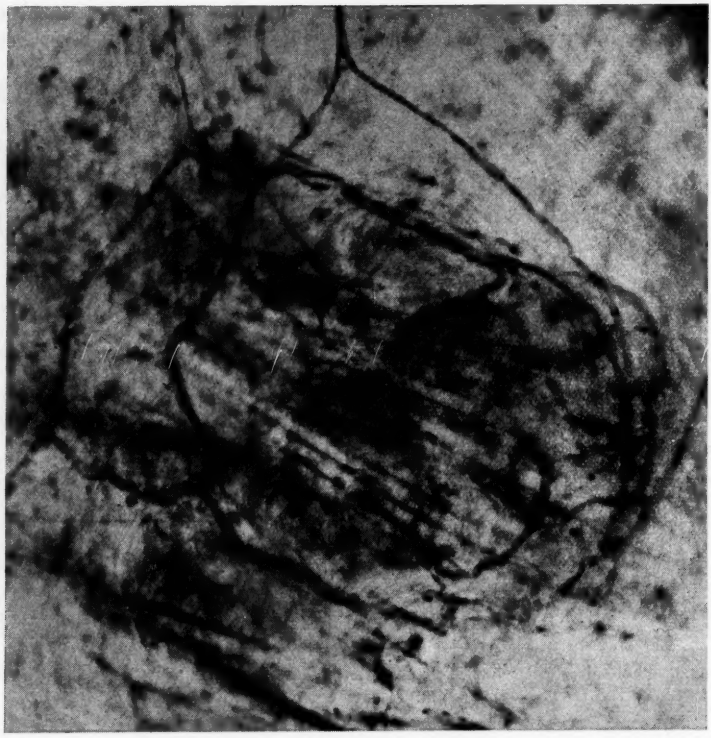
Observations on normal and sympathectomized material. During and for a short time subsequent to introduction of the dye, the skin and subcutaneous tissues of the ear stain uniformly blue. After 15–20 min., nerves and nerve endings close to blood vessels begin to stain specifically. Twenty to thirty min. after injection, the remaining nerves become stained and nerve endings become visible. The colour fades from the surrounding tissues as the nerves become

Text-fig. 5. Photomicrograph showing deep and superficial nerve plexuses in the skin of the ear of the rabbit. Methylene blue. ×33.

stained. Hair follicles and sebaceous glands remain blue for a longer period than other non-nervous tissues (Text-figs. 5, 9 and 10 and Pl. I, fig. 1). The blue begins to disappear from the nerves and nerve endings about 45 min. after the injection of the dye. If large lymphatic vessels are ruptured during the injection, the dye is seen to pass along these channels and no staining of nerve results. Fibroblasts (Pl. I, fig. 1), macrophages, endothelial cells, reticular connective tissue and muscle fibres around vessels stain markedly when the concentration of the dye exceeds 0·02 %. Neurilemma sheath cells frequently stain, but become clearly outlined only when the concentration of the dye exceeds 0·01 %.

In the deeper layers of the dermis there is a nerve plexus (Text-fig. 5 and

Pl. I, fig. 1) consisting of thick and thin medullated and non-medullated fibres. The medullated fibres are sometimes of smaller diameter than the non-medullated fibres. This plexus gives rise to a more superficial plexus consisting of thinner medullated and non-medullated fibres (Text-fig. 5 and Pl. I, fig. 1). The finer fibres often appear varicose. In both plexuses, several axis cylinders of both thick and thin varieties commonly course within a single



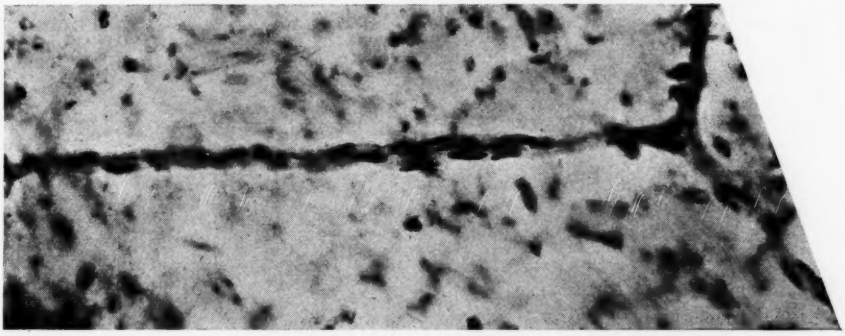
Text-fig. 6. Photomicrograph showing nerve fibres innervating a group of hair follicles. Methylene blue. Rabbit ear. ×470.

neurilemma sheath. The thick fibres supply hair follicles (Text-fig. 6), as many as seven fibres ending on a single follicle; finer medullated fibres from both plexuses may also supply hair follicles (Pl. II, fig. 3). Nerve fibres leave the superficial plexus—the medullated fibres losing their medullary sheaths—assume a varicose appearance, branch repeatedly over a wide area, and end in fine, naked, beaded terminals (Pl. II, fig. 5) disposed below and among the cells of the deeper layers of the epidermis. Similar nerve fibres and nerve endings occur in the adventitia of blood vessels (Pl. I, fig. 2 and Pl. II, fig. 4.)

Non-medullated fibres end on blood capillaries, blood vessels and larger lymphatic vessels by small expansions, a single fibre undergoing a minimum of branching and covering a relatively small area of vessel wall. No encapsulated nerve endings occur in the skin of the ear of the rabbit.

Unilateral cervical sympathetic neurectomies were performed on two rabbits. Persistent Horner's syndromes ensued. After 74 and 21 days respectively, the only nerve fibres and nerve endings not demonstrable were those supplying limited portions of the walls of capillaries, blood vessels and larger lymphatic vessels (Pl. I, figs. 1, 2 and Pl. II, figs. 4, 5).

Stimulation by a fine needle of the stained free nerve endings in the skin, or stimulation previous to section by a spear-headed needle of the stained nerve bundles of the superficial or deep dermal plexus in lightly anaesthetized

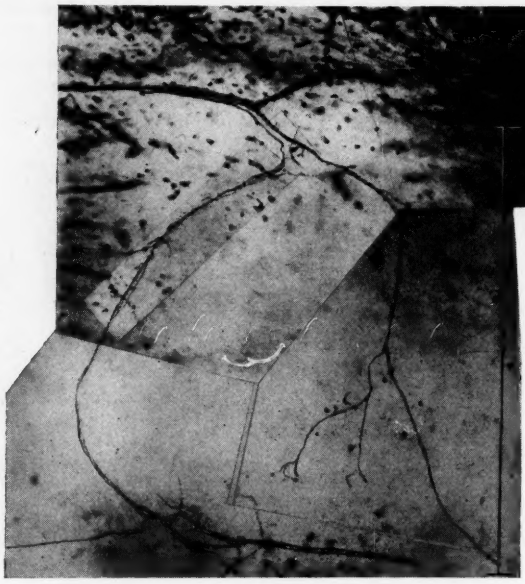


Text-fig. 7. Photomicrograph showing a neurilemmal sheath containing a degenerating fibre. From a denervated area of skin in the ear of a rabbit. Methylene-blue preparation, 2 days after nerve section. ×450.

animals causes struggling. Stimulation of stained nerves passing to, and of the terminal nerve apparatus around, hair follicles calls forth no such reaction.

Experimental observations. Cutaneous nerve bundles consisting of two to four axones were sectioned. On completion of the section, the ends sprang apart, and a small quantity of blue substance exuded from each cut end. A day later, in a continuous area surrounding the site of section, the dye persisted unchanged in intensity of colour for a considerable period of time. The hair follicles were more deeply stained than in normal material, but less clearly outlined, neurilemma sheath cells were enlarged, their nuclei stained more intensely, and nerve fibres within the sheaths were fragmented. Two days later, only the outlines of nerve sheaths were demonstrable (Text-fig. 7). The size of the affected area varied from 2×3 mm. to 10×17 mm. according to the number of axis cylinders in the nerve bundle sectioned. Approximately 5 days after a nerve bundle had been sectioned, two to four regenerating nerve fibres were seen invading the periphery of the denervated area. When the denervated area was small, complete regeneration was found to take place in 8 days.

Examination of the margins of denervated areas revealed that a number of nerve fibres arising from the superficial plexus and a few of the finer fibres from the deep plexus extended farther towards the denervated area than other fibres (Text-fig. 8 and Pl. III, fig. 6) for distances averaging 1 mm. The overlapping somatic fibres from the superficial plexus gave rise to fine beaded naked nerve terminals distributed to the superficial layers of the dermis,



Text-fig. 8. Photomicrograph showing overlap between collaterals of nerve fibres extending towards a denervated area in the skin of the ear of a rabbit. Methylene-blue preparation 3 days after nerve section. ×160.

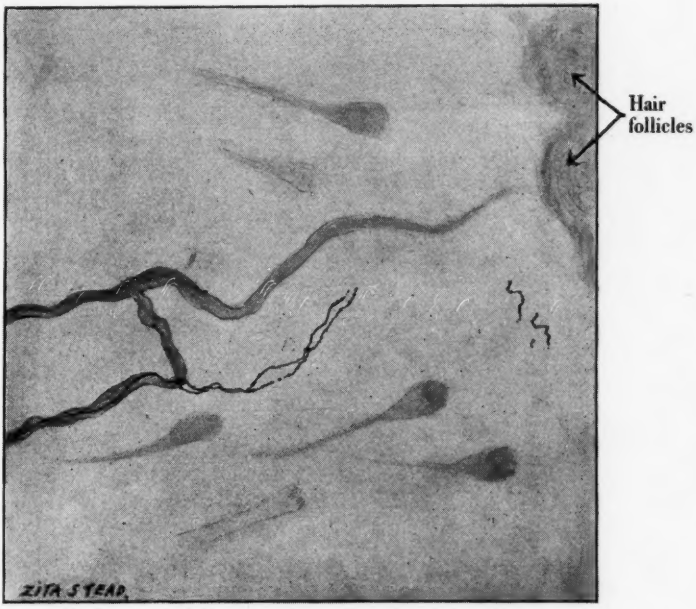
deeper layers of the epidermis, and the adventitia of blood vessels. The thin overlapping fibres often ran for some distance within a sheath common to fragmented degenerating axis cylinders passing to diffusely outlined hair follicles within the denervated area. When axis cylinders supplying hair follicles had degenerated completely, their previous existence could often be inferred from the outline of the persisting neurilemma sheath (Text-fig. 9).

When a fine fibre overlaps towards the denervated area, it sometimes gives off divergent terminal branches (Text-fig. 8 and Pl. III, fig. 6). These branches overlap with similar collaterals from a neighbouring fibre within the marginal

area (Text-fig. 8). The number of nerve fibres bearing free endings in the area of overlap is less than in normally innervated skin.

Stimulation, by pricking with a fine needle, of the overlapping epidermal and subepidermal branched varicose fibres and free nerve endings at the margins of denervated areas, elicited struggling in lightly anaesthetized animals.

Similar observations were made on the margins of artificially produced ulcers on the skin of the rabbit's ear.

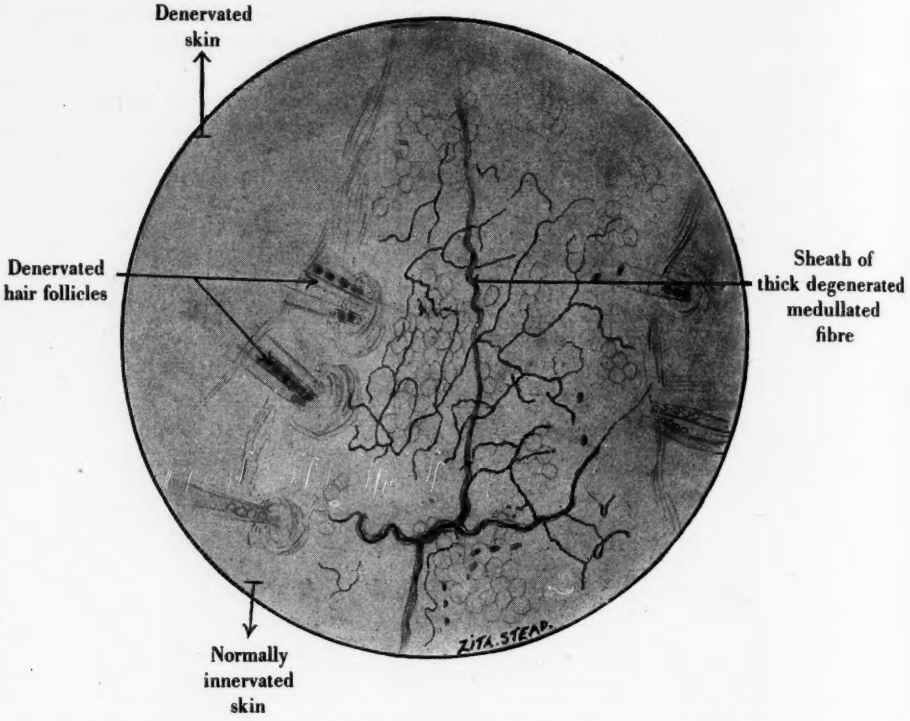


Text-fig. 9. Camera lucida drawing showing a fine medullated nerve fibre giving rise to free nerve endings in an overlap area of skin in the ear of a rabbit. The sheath of a degenerated medullated fibre supplying a hair follicle is outlined. Methylene-blue preparation 3 days after nerve section.

Nerve overlap in the thoracic skin of the rabbit

Two adjoining intercostal nerves were avulsed close to the intervertebral foramina. Ten to twelve days later, preparations were made by perfusion of 0·1 methylene blue in normal saline solution through the aorta. The finer medullated and non-medullated nerve fibres bearing free endings extended for 3–5 mm. from the adjoining normally innervated skin into the affected area. The overlapping nerve fibres and nerve endings have the same morphological characteristics as those at the margins of denervated areas in the ear (Text-

fig. 10). There is a reduction in the number of nerve fibres bearing free endings per unit area in comparison with normal regions.



Text-fig. 10. Camera lucida drawing showing fine nerve fibres and free nerve endings in an overlap area in the thoracic skin of a rabbit. Methylene-blue preparation 10 days after avulsion of two adjacent intercostal nerves.

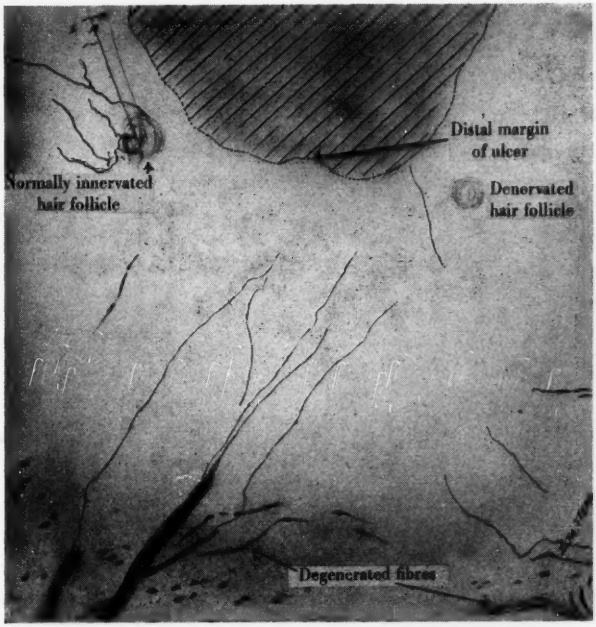
Observations on human skin

Eight days after producing the ulcer on the forearm, 0·02 % methylene blue in normal saline solution was injected into the surrounding skin. After 30 min., the skin around the ulcer was excised, and prepared for histological examination. In the skin immediately distal to the ulcer, only fine nerve fibres occur, giving rise to free subepidermal and intraepidermal nerve endings (Text-fig. 11).

Methylene blue and reduced silver preparations of normal skin from the finger, forearm and thigh were examined. The superficial, subepidermal, nerve plexus and its associated nerve endings are similar to those in the rabbit (Pl. IV, fig. 7 and Text-fig. 12). The intraepidermal nerve endings occupy the deeper layers of the stratum mucosum, except in the fingers where they some-

times extend into the stratum granulosum. In silver material, they occupy an intercellular position and rarely present a varicose appearance. The plexus and nerve endings in question are present in material from the forearm of a patient on whom a "stellate" ganglionectomy had been performed.

"Accessory" nerve endings to Meissner's corpuscles and Krause's end bulbs (Text-fig. 13) were observed in methylene-blue and reduced silver

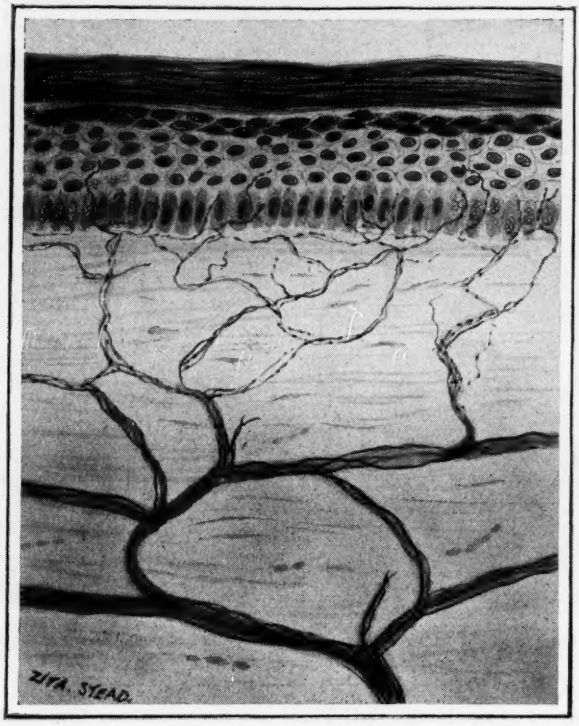


Text-fig. 11. Camera lucida drawing showing the innervation of the skin immediately distal to an ulcer on the human forearm. Methylene blue. ×85.

preparations of normal material. They are derived from fine medullated or non-medullated fibres and course in close proximity to the thick medullated fibres which give rise to the principal nervous components of the endings. The "accessory" fibres often expand somewhat on approaching the ending, and then terminate by again becoming thinner and forming a fine skein within the corpuscle. From the fibre arise free nerve terminals distributed to the surrounding dermal tissues. "Accessory" fibres and endings present a varicose appearance in methylene-blue material and are similar to the fibres and free nerve endings of the subepidermal plexus.

CORRELATION BETWEEN THE HISTOLOGICAL AND PHYSIOLOGICAL FINDINGS

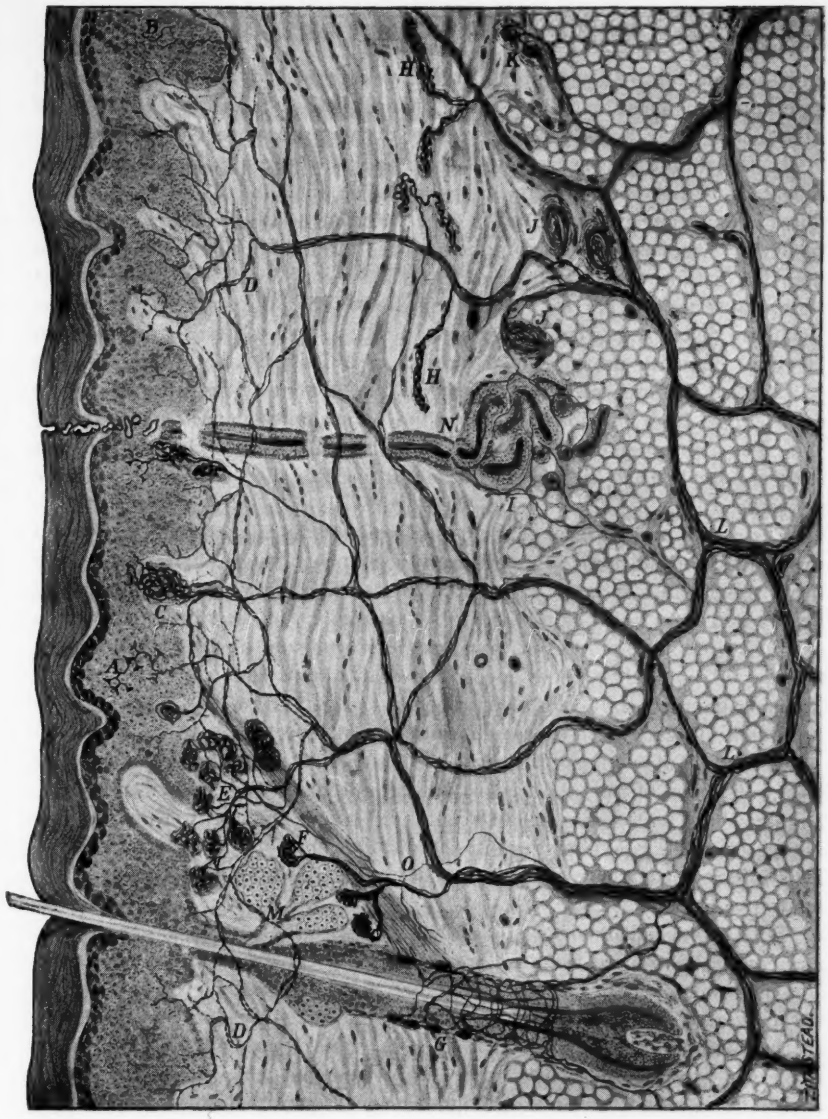
In the skin of the ear of the lightly anaesthetized rabbit, direct stimulation of the finer medullated and non-medullated fibres, and of free nerve endings to which they give rise, leads to struggling. No such reaction results from similar stimulation of the nerve fibres supplying hair follicles. These facts suggest



Text-fig. 12. Drawing showing the arrangement in the skin of the human forearm of nerve fibres and nerve endings subserving pain. Composite drawing from methylene-blue and reduced silver preparations. ×1100.

that the fine nerve fibres and their associated endings subserve pain. From a small area of skin at the distal margin of the ulcer on the human forearm, pain only could be aroused. The neuro-histology of this area establishes that in the human skin pain is subserved by fine nerve fibres bearing free nerve endings.

The nerve fibres bearing free nerve endings extend farther towards denervated areas than other cutaneous nerve fibres and endings in the ear



Text-fig. 13. Composite diagram showing the innervation of the human skin. *A*. Merkel's discs, subserving touch. *B*. Free endings, subserving pain. *C*. Meissner's corpuscles, subserving touch. *D*. Nerve fibres, subserving pain. *E*. Krause's end bulbs, subserving cold. *F*. Nerve endings, subserving warmth (sometimes called Ruffini's endings). *G*. Nerve fibres and endings on hair follicle, subserving touch. *H*. Ruffini's endings, subserving pressure. *I*. Sympathetic nerve fibres innervating a sweat gland. *J*. Pacinian corpuscles, subserving pressure. *K*. Golgi-Mazzoni endings, subserving pressure. *L*. Nerve trunks containing thick and thin fibres. *M*. Sebaceous gland. *N*. Sweat gland. *O*. Sympathetic nerve fibres supplying arrector pili muscle. Drawing composed from methylene-blue and reduced silver preparations. The functional interpretations summarized above are based upon observations by the writers.

and thoracic skin of the rabbit. On the volar aspect of the human forearm and hand, dissociation of sensation on complete local anaesthetization of a cutaneous nerve demonstrates overlap of the nerve apparatus subserving pain.

In the rabbit, the nerve fibres presumably subserving pain dichotomize repeatedly, innervating relatively large areas of skin. The extent to which the fibres in question overlap towards denervated areas is also indicative of the relatively large area of skin supplied by the terminals of one such fibre. In man, the distance to which the nerve fibres and endings subserving pain overlap those subserving other cutaneous sensations is of the same order as the minimum distance at which two simultaneously applied stimuli arousing pain of equal intensity are perceived individually. This distance is as much as 1–1·2 cm. on the forearm. The facts suggest that, in man, a nerve fibre subserving pain derived from one dorsal root ganglion cell supplies a considerable area of skin. Thus, although cutaneous sensation has been found to be punctate, this does not imply that each spot contains a receptor derived from a separate neurone.

The extent and position of the hypoalgesic area in the sensory dissociation experiments on the human forearm, and the neuro-histology of the overlapping nerve fibrés and endings presumably subserving pain at the margins of denervated areas in the skin of the rabbit, indicate that plexiform interlocking systems of fine nerve fibres bearing free nerve endings subserve the sensation of pain in man. The occurrence of spatial summation when two stimuli arousing pain are simultaneously applied at a distance of less than 1 cm. on the human forearm is in agreement with this interpretation.

The extent of overlap of the nerve fibres and endings presumably subserving pain in the skin of the rabbit is greater in the thoracic region than in the ear. In man, the extent of overlap of the cutaneous nerve apparatus mediating pain is greater in the proximal than in the distal regions of the upper extremity. It is inferred that there are corresponding regional differences in the neuro-anatomy in man.

Both superficial and deep pain are localized with the same order of accuracy, and cannot be aroused from the areas affected by antero-lateral chordotomy. The neuro-histology of normal skin in man and in the rabbit, the histology of nerve overlap, and the relative extent of overlap of superficial and deep pain in the human forearm, suggest that both varieties of cutaneous pain are subserved by the same nerve apparatus, deep pain being experienced when several fibres of the nerve plexus in question are stimulated. This interpretation accounts for the greater intensity and diffuse character of deep pain on the basis of spatial summation. It is suggested that the delay in onset and the persistence after stimulation of deep pain are related to the number of nerve fibres stimulated in a single nerve bundle. The differences in the time relations between the two types of pain aroused by a single rapid stimulus in various regions of the body are in accordance with the suggestion of a varying neuro-histology.

Hyperalgesia occurred at the margin of the ulcer on the human forearm. In a hyperalgesic area of skin immediately distal to the ulcer, pain only could be aroused, and the neuro-histology of this area established that only one type of nerve fibre and associated ending was present. Hyperalgesia is therefore associated with no nerves and endings other than those subserving pain.

DISCUSSION

Blix (1884, 1885), Donaldson (1885), v. Frey (1894, 1895, 1896, 1897, 1926), Goldscheider (1886, 1898), Sherrington (1900), Sommer (1900), Rivers & Head (1908), Trotter & Davies (1909), Haggqvist (1914), Strughold (1923), Foerster (1927), Dallenbach (1927), Pendleton (1928) and Waterston (1923, 1933 *a*, *b*) have brought forward evidence in support of the punctate theory of cutaneous sensation. The present findings, in conjunction with unpublished histological observations summarized in Text-fig. 13, and the findings of Strughold & Karbe (1925), Bazett (1935) and Woollard (1935, 1936*a*) concur in allocating specific sensory functions to morphologically characteristic nerve endings. The findings of the present writers as to the relative depths at which the various specific receptors are located in the skin of the human forearm are in agreement with the observations of Williams (1935), working on the human prepuce. The nature of the sensations aroused by the introduction of a needle depends upon both the spot at, and the depth to which, the needle is introduced (Text-fig. 13). Thus the theory of punctate sensation must be considered from a three-dimensional point of view. Waterston's (1923) finding that warmth and cold spots vary in position at different times is explained by the fact that the receptors concerned lie deep in the skin and are influenced by the state of the local blood vessels as well as by the external temperature. Burnett & Dallenbach (1927) have demonstrated that the feeling of heat is a complex of warmth and paradoxical cold with or without pain. The anatomical basis for this conception is implicit in a three-dimensional theory of punctate sensibility.

The large number of pain spots in the skin has made it difficult to establish the punctate representation of this sensation. v. Frey (1894), by the use of bristles, was able to evoke pain without an accompanying sensation of contact. Foerster (1927), from observations on blistered skin, has concluded that cutaneous pain is punctate. The same conclusion was reached by Rivers & Head (1908) and Foerster (1927) from observations on cutaneous areas the nerves to which were in process of regeneration. Waterston (1933 *a*, *b*) has found that in the skin there are small analgesic areas, and describes cutaneous pain as being punctate. Blix (1885) has denied that pain is represented in the skin in a punctate form analogous to warmth and cold spots. v. Frey (1899) states that every nerve ending is distinct from every other nerve ending and that it is not possible to distinguish distances of sensation on the skin smaller than the distance between two nerve endings. Tower (1935) states that in the cornea of the cat a single nerve fibre supplies an area of $\frac{1}{2}$ sq. cm. or more. The

findings recorded in the present paper show that cutaneous pain can be provoked from constant spots, but that many such spots occur within the relatively large area of skin supplied by one axone.

Waterston (1933*a*, *b*) states that the introduction of a needle into the skin elicits no pain until the needle enters the superficial layers of the dermis, and that shaving off thin slices of the epidermis arouses no pain until the dermis is reached. Waterston considers that only touch can be aroused from the epidermis. Woollard (1936*a*) has correlated histological observations with the results of shaving off thin slices of skin, and has concluded that in man the receptors for pain occur mainly immediately below the epidermis, and only occasionally in the epidermis. The present observations concerning the depths at which various modalities of sensation are aroused show that pain can be aroused from the deeper layers of the epidermis. Shaving off slices of skin is too crude a method of testing for epidermal pain.

Ranson (1913, 1914, 1931) has shown that the centripetal processes of the dorsal root ganglion cells which give rise to peripheral fine medullated and non-medullated fibres, lie in the lateral part of the dorsal roots and enter the tract of Lissauer. Ranson & Billingsley (1916) have shown that in the cat, stimulation of the portion of the dorsal roots containing the fine medullated and non-medullated nerve fibres leads to struggling, a rise in blood pressure and rapid respiration. These reactions are interpreted as resulting from a sensation of pain. Gasser & Erlanger (1929), Gasser (1935), Heinbecker *et al.* (1934) and Clark *et al.* (1935), from studies with the cathode-ray oscilloscope, have shown that pain is transmitted by slowly conducting fine medullated and non-medullated nerve fibres. The observations recorded in the present paper are in agreement with such conclusions.

Ranson *et al.* (1935) have indicated that there is no sharp distinction in function between fine medullated and non-medullated nerve fibres. There are individual and species variations in the relative numbers of these types of fibre in the same nerve or nerve root. The proportion of medullated fibres is greater in larger animals, in which the diameter of nerve fibres is in general greater. These findings are stated to be in agreement with Duncan's (1934) theory of progressive myelinization with increasing diameter of nerve fibres. Observations by Weddell *et al.* (1940) on the innervation of the musculature of the tongue, and the present study of the innervation of the skin, show that whilst medullated nerve fibres commonly lose their myelin sheaths on becoming finer in diameter, medullated nerve fibres are sometimes of smaller diameter than non-medullated fibres.

Adrian (1926, 1932) has shown that in the frog the impulses produced by painful stimuli are distinct from those produced by touch. Heinbecker & Bishop (1935) state that stimulation of nerve fibres conducting tactile or pressure sensation never results in pain. Heinbecker *et al.* (1934) have claimed that near-threshold stimulation of the fibre group conducting pain results in a sensation of pricking touch. In their experiments the possibility that tactile

receptors were being stimulated at the same time as pain receptors was not satisfactorily excluded. In the present experiments no sensation other than pain could be provoked from overlap areas. The evidence indicates that nerve-fibres transmitting pain are specific in this respect.

Cattell & Hoagland (1931) have shown that in the frog, no reactions that can be interpreted as resulting from pain take place when tactile receptors are caused to discharge impulses at very high frequency. Adrian (1932) has shown that in mammals pain is not provoked by high-frequency impulses from hair receptors. Thus pain is not aroused by overstimulation of these tactile receptors. Heinbecker & Bishop (1935) state that nerve endings subserving pain contrast with tactile receptors in having a relatively lower threshold for electrical than for mechanical stimuli. They interpret this finding as suggesting that electrical stimulation of pain receptors is essentially nerve-fibre stimulation, which in turn suggests the non-encapsulated character of these endings. Goldscheider (1926) considers that pain may result from excessive stimulation of endings recording deformation. Timofeyev (1895), Ruffini (1902), Dogiel (1892, 1893, 1904), Sokolow (1899), Sala (1899), Sfameni (1901), Michailow (1908), Ohmori (1923), Lalowy (1935), Woollard (1936*b*, 1937), Weddell & Harpman (1940), and others have described "accessory" nerve fibres and endings to encapsulated touch receptors, and to nerve endings subserving pressure, cold and proprioception from tendon. Lavrenko (1938) has established that the "accessory" fibres are somatic in origin. The "accessory" nerve fibres and endings are morphologically similar to the nerve fibres and terminals subserving pain. Woollard (1937) has described "accessory" fibres derived from the subepidermal nerve plexus.

v. Frey (1925) and Strughold & Karbe (1925) state that stimulation of the central part of the cornea arouses pain only. Nagel (1895), Marx (1921) and Pritchard (1931) remark that after cocainization of the conjunctiva a sensation of contact can be evoked from the cornea. This finding is explicable on the grounds of stimulation of extra-corneal sensory nerve endings by deformation. This explanation is suggested by the findings of Carmichael & Woollard (1933). The innervation of the cornea has been described by Ranvier (1878), Dogiel (1890), Attias (1912), Agabow (1912), Cajal (1933) and others. Medullated nerve fibres of small calibre reach the cornea *via* the sclera and ciliary body. The majority lose their myelin sheaths on entering the cornea. At times, several non-medullated nerve fibres lie within a single sheath (Nageotte, 1932). The nerve fibres form plexuses at various levels in the corneal connective tissues. In methylene blue and gold chloride preparations, the nerve fibres often present a varicose appearance. The fibres become smaller in diameter on approaching the epithelium. Free varicose nerve endings extend among the epithelial cells. The innervation of the cornea is thus similar to the subepidermal and intraepidermal nerve apparatus consisting of fine nerve fibres and free nerve terminals.

It is generally accepted that from teeth, as opposed to periodontal tissues, pain only can be provoked. Nerve fibres which are mostly medullated enter

the pulp cavity and here form a plexus from which arises a more peripherally situated plexus of finer non-medullated fibres (Schafer, 1934; Maximow & Bloom, 1938) bearing free nerve endings distributed to the pulp, dentinal tubules and odontogenic zone (Lewinsky & Stewart, 1938*a*). Free nerve terminals only have been described in the tympanic membrane (Wilson, 1911), from which pain only can be provoked (Clark, 1939). Similar nerve endings derived from fine somatic nerve fibres occur in mucous membranes (Retzius, 1908; Huber, 1900; Cajal, 1933; Kadanoff, 1927; Lewinsky & Stewart, 1938*b*, 1939; Stewart & Lewinsky, 1939), serous membranes (Dogiel, 1902; Timofeyev, 1902), intermuscular connective tissue (Hinsey, 1927, 1930), adventitia of blood vessels (Woollard, 1926; Hinsey, 1927, 1928, 1930; present writers), tendon and periosteum (Weddell & Harpman, 1940), from all of which pain can be aroused (Foerster, 1927; Capps & Coleman, 1932; Waterston, 1933*b*; Lewis, 1938; Kellgren, 1938; Lewis & Kellgren, 1939; and others). Weddell & Harpman have found in the tongue of a rabbit, on which a cervical sympathetic neurectomy had been performed, a subepithelial plexus of fine nerve fibres giving rise to free nerve endings similar to that occurring in the skin (Pl. IV, fig. 8).

Free intraepidermal nerve endings have been described in many vertebrates (Langerhans, 1868; Ranvier, 1878; Merkel, 1880; Retzius, 1892; Dogiel, 1893; v. Gehuchten, 1906; Bielschowsky, 1907; Botezat, 1908, 1912; Cajal, 1909, 1933; Ackert, 1914; Ohmori, 1923; Kadanoff, 1924, 1928, 1929–30; Ludford, 1930; Lalowy, 1935; Woollard, 1936*a*; and others). In general, they are abundant, derived from medullated and non-medullated fibres, and sometimes extend into the stratum granulosum. Intraepidermal free nerve endings extending into the stratum granulosum have been seen by the present writers in the human finger, but not in skin from the human forearm or thigh. No general agreement has been reached as to whether intraepithelial free nerve endings are intra- or extracellular in position (Boeke, 1932). In the sections examined by the present writers, however, the intraepidermal free nerve endings are extracellular in position.

In methylene-blue preparations fine nerve fibres and free nerve terminals commonly present a varicose appearance, whilst in material impregnated with silver they rarely appear varicose. Woollard & Harpman (1939) have suggested that in coelenterates the large varicosities are due to toxic effects of methylene blue upon nerve fibres. Strughold & Karbe (1925) studied the innervation of the human cornea and conjunctiva *in vivo*, by the instillation of 0·01–1 % methylene-blue solutions. They found that introduction of the dye at first caused intense pain, followed by analgesia and anaesthesia to cold; the receptors concerned with the latter sensation were more resistant to the effects of the dye. In the present experiments stimulation by needle pricks of cutaneous nerve fibres and free endings stained with methylene blue in the living rabbit caused struggling.

There has been much controversy concerning the problem of syncytial

continuity in the nervous system. Many discrepant descriptive observations have been made on the cutaneous nerve plexuses (Dogiel, 1890, 1893, 1904, 1905; Ruffini; Ohmori, 1923; Tello, 1932; Woollard, 1936*a*, 1937). The occurrence of degenerating along with normal axis cylinders within one sheath after nerve section in the ear of the rabbit suggests that the nerve plexuses in question are not a neurencytium.

Foerster (1925, 1927) has recorded the occurrence of pain from stimulation of the distal ends of sectioned cutaneous nerves in the human subject. The pain is abolished by section of adjacent overlapping cutaneous nerves. The anatomical basis for this phenomenon is the fact that nerve fibres and endings subserving pain form closely interlocked systems.

Lewis & Pochin (1937) have confirmed and expanded observations by Rosenbach (1884), Gad & Goldscheider (1892), Thunberg (1902) and Alrutz (1909), showing that from human skin two pain sensations can be aroused by a single stimulus. On the volar aspect of the forearm or dorsum of the finger, "light pricks" arouse a second pain which is more intense, diffuse and longer lasting than the first pain, which is frequently not felt. "Heavier jabs" evoke a first pain which is more intense than the second pain. Application of a hot metal cylinder 1 cm. in diameter for $\frac{1}{8}$–$\frac{1}{2}$ a second is accompanied by a brief pain, followed after an interval, during which the metal has been removed, by a second pain which, "if the contact has been short", exceeds the first in intensity. The type of pain provoked, therefore, depends upon the stimulus used. It is noteworthy that the "most certain method" of evoking the double pain sensation was the application of hot metal cylinders 1 cm. in diameter.

Lewis & Pochin (1937) have demonstrated that in man the interval between the two pain sensations diminishes as skin nearer the spinal cord is tested. The margin at which the two cutaneous pain sensations fuse is stated to be at an approximately constant distance from the spinal cord, if measurements be made along the corresponding nerve trunks. The present workers have found that by using fine needles inserted rapidly and deeply, two pain sensations separated by an interval can be aroused from the skin covering the extremities, face, forehead, neck, upper part of the chest and shoulders, from which regions, however, a phasic sensation can also be provoked. From the skin of the back or abdomen, phasic sensations only can be provoked. Lewis & Pochin (1937) have also recorded that although hot metal contacts evoke a single pain sensation from the face, a needle prick is "often followed by a second sensation, which comes gradually, and lasts for very many seconds. It is a little sense of continuous sting, the nature of which we have not investigated, but which is to be distinguished from the fleeting pain of a second response, as this is felt in finger or toe." The present writers are, however, unable to detect a fundamental distinction between the second pain sensation aroused from the skin of the face and that provoked from the skin of other regions of the body, thus agreeing with Lewis's later (1938) statement that cutaneous pain of only one "quality" can be provoked from the skin. The present observations

indicate that there are regional differences in the extent of skin supplied by one dorsal root ganglion cell subserving pain. This inference is supported by the fact that the number of nerve fibres supplying similar areas of skin varies in different regions of the body (Ingbert, 1903; Ranson *et al.* 1935), and the fact that section of a dorsal root may or may not produce an area of cutaneous anaesthesia according to the region from which it derives its fibres (Sherrington, 1893, 1898; Foerster, 1933, 1936). A hot metal cylinder 1 cm. in diameter applied to the skin would stimulate a different number, or a different relative distribution of components, of discrete terminal nerve plexuses subserving pain, derived from single dorsal root ganglion cells, in different regions of the body.

Lewis & Pochin (1937) state that in man the two varieties of cutaneous pain can be dissociated from each other by asphyxia or cocainization of a cutaneous nerve. The same authors later (1938) state that "in asphyxia of a limb, defects of touch, cold sense, warm sense and fast conducted pain begin almost simultaneously. The defect in slow conducted pain comes later, and is preceded by exaggerated pain response. The only sense to be lost early is touch; the rest all decline slowly, and together are eliminated within a relatively short time period of each other. The declines of fast and slow conducted pain do not occupy separate phases of asphyxia, they overlap considerably. In asphyxia of a limb, the separate sensory functions are not eliminated in a simple precise order, neither do nerve fibres fall out in the order of their conduction rates." Gasser (1935), from a review of the literature and cathode-ray oscilloscope studies, concludes that the fibres conducting different modalities of sensation are widely distributed throughout the various fibre sizes. The observation that the diameter of fibres supplying hair follicles in the skin of the ear of the rabbit is sometimes less than that of fibres from the nerve apparatus presumed to subserve pain lends support to the conclusions of Lewis & Pochin (1938) and Gasser (1935). The fact that asphyxia does not eliminate separate sensory functions "in a simple precise order", and the fact that nerve trunk asphyxiation does not cause nerve fibres to "fall out in the order of their conduction rates", suggest that nerve trunk asphyxiation might have the same effect as an alteration of the anatomical relationships between the plexiform closely interlocking nerve fibres and endings subserving pain. The present writers, therefore, are of the opinion that the findings of Lewis & Pochin may equally well be explained on a basis of regional differences in the relationships, either present anatomically or induced by asphyxia, between the plexiform interlocking fibres and endings subserving pain. The histological observations here recorded show that there are not two sharply separated groups of fibre sizes mediating two varieties of cutaneous pain, but that fibres heterogeneously distributed through a spectrum of sizes bear endings of a single morphological type.

Lewis (1936, 1937*a*) has brought forward evidence showing that cutaneous hyperalgesia, flare, whealing and antidromic dorsal root vasodilatation are

probably mediated by a single system of nerves, which he has designated "nocifensor". Lewis (1937*b*) states that asphyxia of the skin prevents the development of hyperalgesia by paralysing "nocifensor" nerves; these nerves are paralysed before the sensory nerves. Asphyxia must be carried a stage further to prevent the development of flare reaction. Cocaine introduced electrophoretically into the skin paralyses nerves subserving pain completely, and nerves subserving touch entirely or almost entirely, before it interferes with the hyperalgesia reaction. Hinsey & Gasser (1930), from studies using the cathode-ray oscilloscope, have determined that vasodilatation due to dorsal root stimulation is mediated by slowly conducting fibres. Heinbecker & Bishop (1935) state that fibres conducting pain evoked from the skin have a lower threshold and a faster conduction rate than autonomic fibres of similar morphological type and size. The present observations show that hyperalgesia occurs in skin supplied only by nerves and nerve endings subserving pain. The observations of Hinsey & Gasser, and Heinbecker & Bishop, suggest that the interpretation of the results of dissociation experiments using asphyxia or cocaine must be accepted with caution. The plexiform closely interlocked nerves and endings subserving pain are anatomically similar to the complex and branching system of axones postulated by Lewis (1936, 1937*a*) as being necessary to explain the phenomena of hyperalgesia, flare, whealing and vasodilatation. The findings with regard to hyperalgesia, and the impossibility of demonstrating histologically several systems of plexiform interlocking fibres, establish that these reactions must take place within the one demonstrable system subserving pain.

Trotter & Davies (1909) postulated a chemical factor to explain the mechanism of hyperalgesia occurring in nerve regeneration. Lewis (1933) and Lewis & Hess (1933) conclude that after-sensations of pain are dependent upon the liberation by injury of some chemical irritant to the nerves, since this after-sensation of pain may be modified by changes in the circulation. Bazett (1935) suggests that the persisting itching sensation which follows light touch, particularly in the neighbourhood of orifices (v. Frey & Rein, 1929), is due to a chemical factor. Feng (1933) has shown that the balance of excitability between receptors for touch and those for pain may be upset peripherally by the liberation by injury of simple chemical substances such as potassium. Lewis (1936) has found that extracts of human or guinea-pig skin, when injected in small quantities intradermally, after a short delay give rise to smarting which increases in intensity. The smarting is sometimes accompanied by a sensation of itching. Hyperalgesia soon develops around the site at which the juice was injected. A wheal forms at the site of injection. Lewis (1936, 1937*a*) believes that a pain-producing substance is released in the skin, either from direct injury of the skin or through the action of "nocifensor" nerves. The "nocifensor" nerves, it is suggested, cause the release of a substance from skin cells; this substance produces hyperalgesia, itching, and vasodilatation. The present writers suggest that damage to the skin injures the terminal

nerve apparatus subserving pain, and that the impulses thus set up not only pass to the central nervous system resulting in a sensation of pain, but also spread within the terminal nerve plexus derived from one dorsal root ganglion cell. The ionic shifts consequent upon the passage of impulses throughout the discrete plexuses of interlocking nerve fibres and endings subserving pain may cause either chemical changes in the immediate neighbourhood of the free nerve endings arising from the plexuses in question, or cause cells in close proximity to such endings to liberate chemical substances. Such substances, in addition to those liberated by tissue injury, may in turn cause neighbouring closely interlocked fibres to discharge in a similar manner. Limited local spread could thus take place without the necessity of invoking the presence of a neurencytium, and in addition would explain whealing, flare and hyperalgesia. Sensory pain nerves could in this manner subserve all "nocifensor" reactions as well as transmit the sensation of pain. The present observations show that in the skin there is no anatomical foundation for the usual conception with regard to "axone reflexes".

The evidence presented in the present paper, like most modern investigations (Lanier, 1935), offers no support for Head's (1920) theory of protopathic and epicritic systems of fibres in cutaneous nerves.

CONCLUSIONS

1. Pressure, warmth, touch, cold and pain are represented in the skin in punctate form. The theory of punctate sensibility must be interpreted from a three-dimensional point of view. Multiple spots from which one of the primary modalities of sensation can be aroused occur within the area of skin supplied by a single specific sensory neurone.

2. Cutaneous pain is subserved only by the finer medullated and non-medullated nerve fibres bearing free endings. These fibres and endings are specific and arranged in a plexiform interlocking manner.

3. Non-medullated nerve fibres derived from different neurones often lie within the same neurilemma sheath.

4. There are regional differences in the disposition of, and in the area supplied by, the terminal nerve plexus and endings derived from a single dorsal root ganglion cell subserving pain.

5. Pain can be aroused from the deeper layers of the epidermis and the superficial layers of the dermis.

6. Several varieties of cutaneous pain can be aroused by a single stimulus, depending upon the nature of the stimulus and the region of the body stimulated.

7. All varieties of cutaneous pain are subserved by the same nerve apparatus.

8. The "accessory" innervation to encapsulated touch receptors and to nerve endings subserving pressure, cold, and proprioception from tendon, is morphologically similar to nerve fibres and endings subserving pain.

9. Fine somatic nerve fibres bearing free nerve endings similar to those subserving pain in the skin occur in the adventitia of blood vessels.

10. No nerve apparatus other than that subserving pain is involved in the production of hyperalgesia. "Nocifensor" reactions are mediated by the nerve apparatus subserving pain.

11. There is no anatomical foundation for the usual conception with regard to "axone reflexes".

12. The facts presented establish that cutaneous pain behaves in accordance with the doctrine of specific nervous activity.

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EXPLANATION OF PLATES I–IV

All figures are photomicrographs of methylene-blue preparations

Plate I

Fig. 1. Deep and superficial cutaneous nerve plexuses in the ear of a rabbit. A cervical sympathetic neurectomy had been performed 74 days previously. ×80.

Fig. 2. Fine non-medullated nerve fibres and free nerve endings in the adventitia of an artery in the ear of a rabbit. A cervical sympathetic neurectomy had been performed 74 days previously. Nuclei of neurilemma sheath cells surrounding non-medullated nerve fibres are stained. ×260.

Plate II

Fig. 3. The innervation of a hair follicle in the ear of a rabbit. ×600.

Fig. 4. A fine medullated nerve fibre giving rise to free nerve endings in the adventitia of an artery in the ear of a rabbit. A cervical sympathetic neurectomy had been performed 21 days previously. ×320.

Fig. 5. Free nerve endings arising from a fine medullated nerve fibre in the skin of the ear of a rabbit. A cervical sympathetic neurectomy had been performed 74 days previously. The neurilemma sheath cells are darkly stained. ×260.

Plate III

Fig. 6. A cutaneous nerve fibre, bearing free nerve endings, overlapping towards a denervated area in the ear of the rabbit. Preparation made 3 days after nerve section. ×500.

Plate IV

Fig. 7. The subepidermal nerve plexus subserving pain in the human forearm. ×370.

Fig. 8. A plexus of fine nerve fibres, giving rise to free nerve endings, immediately subjacent to the mucous membrane of the tongue of a rabbit. A cervical sympathetic neurectomy had been performed 74 days previously. ×320.

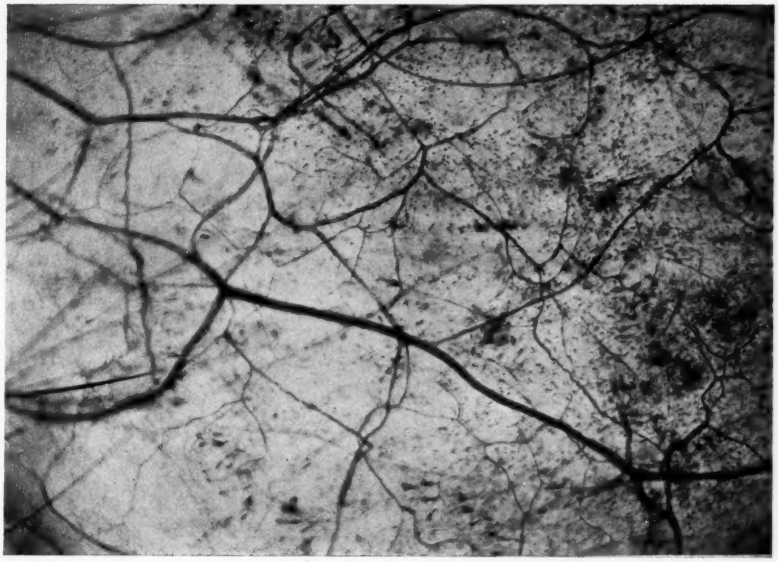

Fig. 1.

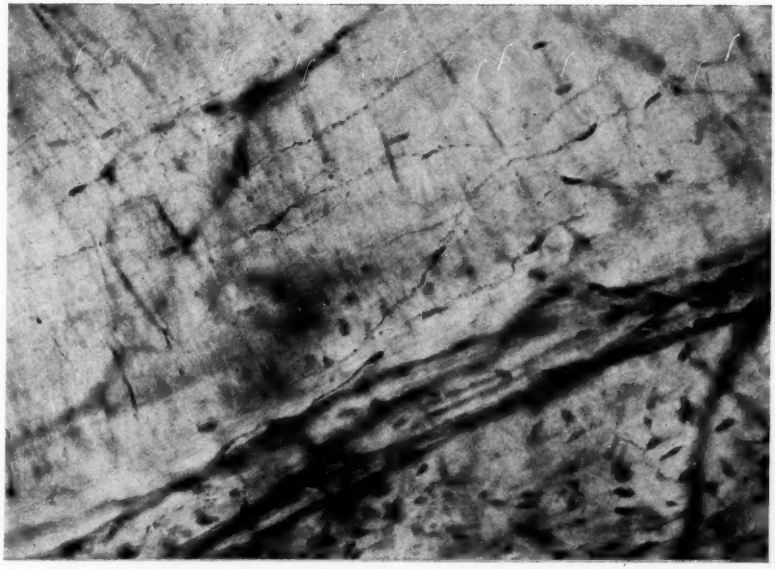

Fig. 2.

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Fig. 3.

Fig. 4.

Fig. 5.

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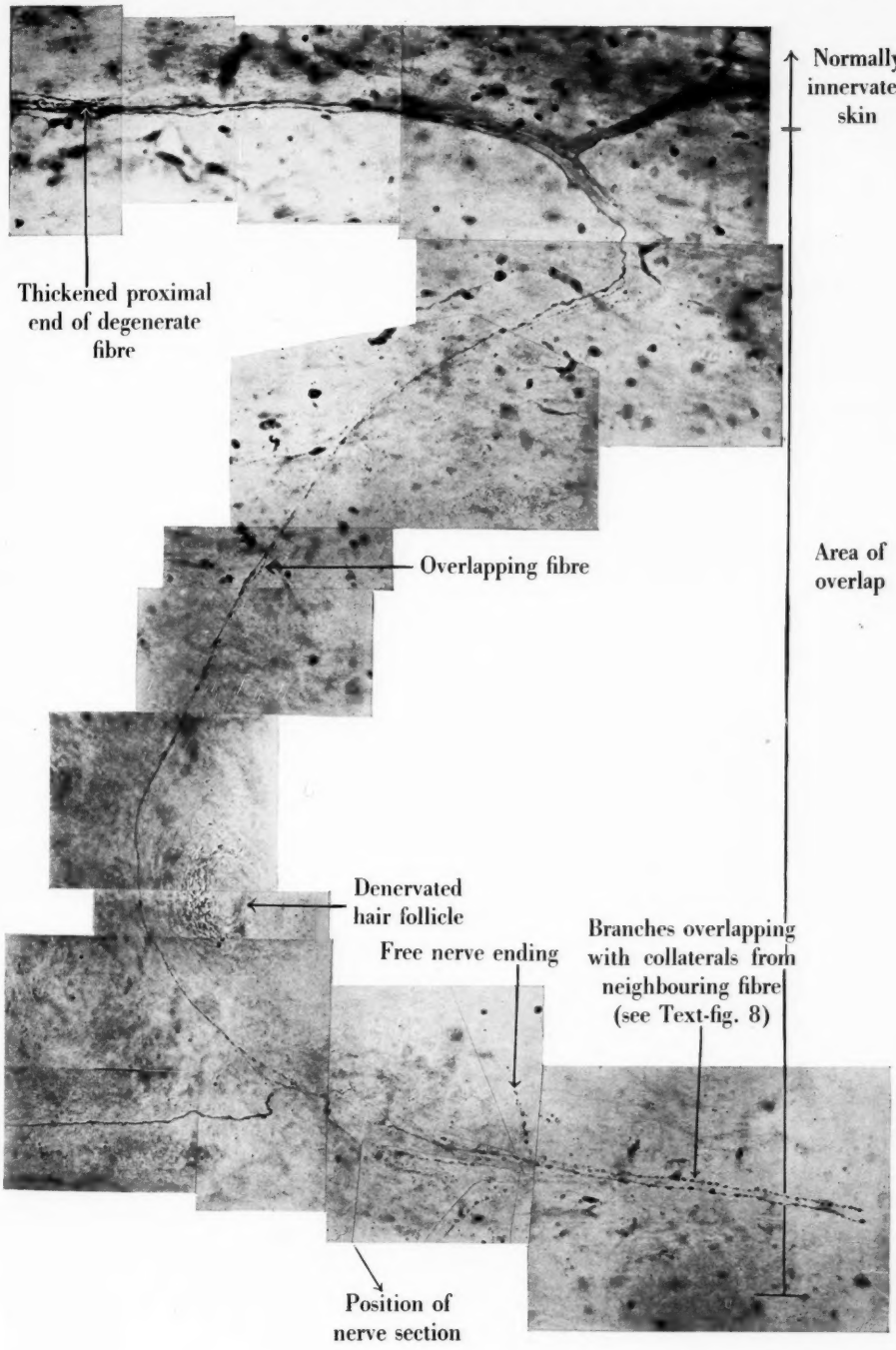
Normally innervated skin
Thickened proximal end of degenerate fibre
Overlapping fibre
Area of overlap
Denervated hair follicle
Free nerve ending
Branches overlapping with collaterals from neighbouring fibre (see Text-fig. 8)
Position of nerve section

Fig. 6.

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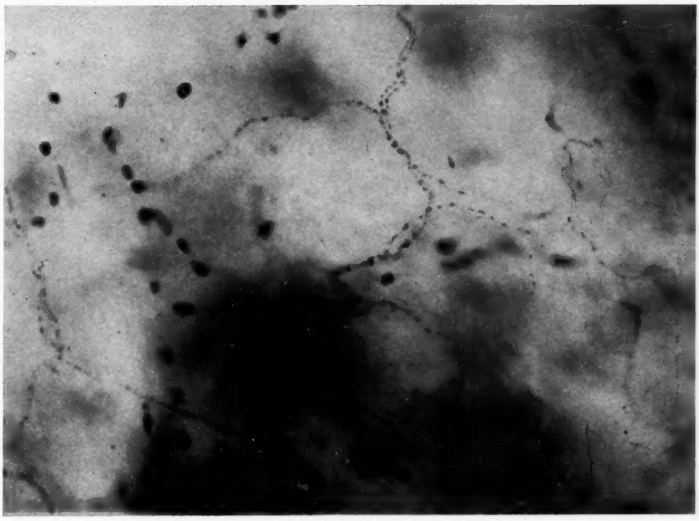

Fig. 7.

Fig. 8.

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THE CONNEXIONS OF THE INFERIOR COLLICULUS AND OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS

By the late H. H. WOOLLARD and J. A. HARPMAN

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The midbrain auditory centres have been the subject of repeated anatomical and physiological investigations. Controversy has ranged around the problems of their fibre connexions and functions. The present paper deals with anatomical observations made with a view to establishing the afferent and efferent connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus. The physiological significance of the anatomical findings is discussed.

MATERIAL AND METHODS

Four guinea-pigs and four cats were used. Lesions were placed in various parts of the medulla or midbrain, by means of a fine knife in the guinea-pigs, and electrolytically by the Clarke-Horsley stereotaxic instrument in the cats. Ether anaesthesia was used, and aseptic precautions were observed. The animals were killed 12 days after the operations. The brains were fixed by perfusion through the blood vessels and prepared by the method of Marchi after fixation in either Müller's fluid or formalin, or according to the chlorate-osmic-formalin technique of Swank & Davenport (1935). The latter method gives preparations in which the incidence of pseudo-Marchi reaction is reduced to a minimum, and allows of counterstaining by cresyl violet.

EXPERIMENTS AND OBSERVATIONS

Experiment 1

In a guinea-pig a lesion was made with the intention of destroying the cochlear nuclei. During recovery the animal exhibited symptoms of interference with the vestibular apparatus.

Histological examination

Lesion. The ventral cochlear nucleus has been cut away and the dorsal cochlear nucleus has been destroyed (Pl. I, fig. 1). The vestibular nerve has been considerably injured. The dorsal spino-cerebellar tract and restiform body have been slightly damaged where they enter the cerebellum. The cranio-ventral portion of the flocculus has also been affected.

Fibre degeneration. Degenerate fibres from the vestibular nerve run medially and dorsally and are distributed to the lateral, medial, and superior vestibular

nuclei (Pl. I, fig. 1). There is some degeneration in the ramus descendens of the vestibular nerve.

From the region of the lesion degenerate fibres pass into the cerebellum and end in the flocculus, uvula, roof nuclei, and in the lobus medius, ansiformis, and paramedianus. The degeneration in the cerebellum is restricted to the operated side except in the region of the roof nuclei and central part of the lobus medius. A set of degenerate fibres runs with the tractus uncinatus of Russell, ending in the homolateral medial vestibular and globose nuclei.

There is massive degeneration in the trapezoid body. The majority of these fibres run along the lateral and ventral aspects of the medulla and lie ventral to the superior olivary nuclear complex of the injured side (Pl. I, fig. 1). A few fibres (fibres of Held) run dorsal to the restiform body and the spinal nucleus and tract of the trigeminal nerve, then turn ventro-laterally, traverse the spinal nucleus of the fifth cranial nerve and join the trapezoid body. One such fibre is shown in Pl. I, fig. 1.

On the side of the lesion some fibres of the trapezoid body end directly in the lateral region of the superior olive. Many collaterals arising from the trapezoid body run dorsalwards and terminate in the superior and accessory olivary and medial praeolivary nuclei. Other fibres and collaterals end in the trapezoid nuclei. The trapezoid body acquires a more dorsal position on crossing over to the opposite side, where similar connexions obtain. The fibre relationships in question have been confirmed in rat brains prepared by a Cajal reduced silver method and Bodian's protargol technique. The synaptic terminations of fibres and collaterals are verifiable in such material.

Degeneration occurs in the acoustic stria. These fibres, from the region of the lesion, pass dorsal to the restiform body and lateral vestibular nucleus, and thence through the ventral region of the superior vestibular nucleus and medial vestibular nucleus to reach a position immediately lateral to the genu of the facial nerve (Pl. I, fig. 1). Here the acoustic stria divides into two parts. Homolateral fibres run ventralwards, medial to the outgoing fibres of the facial nerve, to reach the area of Monakow and end in the ipsilateral superior olivary nuclear complex. A contralateral set of fibres runs diagonally across the medulla to reach the area of Monakow and the superior and accessory olivary nuclei of the opposite side. Some fibres of the acoustic stria may end in the formatio reticularis on either side of the raphe. Possibly a few fibres of the stria acoustica contribute to the lateral fillet. A group of fibres crosses in the dorsal half of the medulla and ends in the formatio reticularis lateralis, between the spinal root of the trigeminal and the outgoing fibres of the facial nerve. The origin of these fibres is uncertain.

A small number of degenerate fibres runs in the lateral fillet of the operated side; a few end in the corresponding nuclei of the lateral fillet, the remainder ending in the inferior colliculus. The lateral fillet of the opposite side contains many degenerate fibres (Pl. II, fig. 1). Some fibres end in the ventral and dorsal nuclei of the lateral fillet, and a few collaterals form part of the dorsal com-

missure of the lateral lemniscus. The majority enter the inferior colliculus, to the central nucleus of which they are distributed (Pl. II, fig. 1).

It is possible that from their position in the central nucleus of the inferior colliculus some of the degenerate fibres turn horizontally cranialwards to end in the three deeper layers of the superior colliculus. A small number of lateral fillet fibres courses in the inferior brachium and ends in the medial geniculate body.

Experiment 2

In a guinea-pig a lesion was made in the caudal and dorso-lateral region of the midbrain, a small amount of superficial tissue being removed. The animal made a good recovery.

Histological examination

Lesion. The anterior two-thirds of the right inferior colliculus have been injured (Pl. II, fig. 2). The lesion extends to the medial border of the colliculus, and cranially involves the caudal part of the superior colliculus. On the lateral aspect of the midbrain the lesion extends ventrally, affecting the lateral region of the dorsal nucleus of the lateral fillet and nucleus parabigeminus.

Fibre degeneration. There is much degeneration in the right inferior brachium, the fibres of which terminate in the caudo-ventral nucleus of the medial geniculate body. Possibly a few fibres end in the other nuclei of the medial geniculate body.

The inferior intercollicular commissure contains many degenerate fibres, which end in the dorsal half of the inferior colliculus of the opposite side.

A bundle of thick fibres arises from the ventro-lateral aspect of the inferior colliculus and courses medialwards between its ventral border and the dorsal nucleus of the brachium conjunctivum (Pl. II, fig. 2). Some of the fibres enter the central grey matter of the aqueduct of Sylvius. Others extend no further than the mesencephalic nucleus of the trigeminal nerve, and possibly terminate in it. The terminations of these fibres could not be established by the examination of reduced silver preparations.

Tecto-pontine fibres arise from the region affected by the cranial part of the lesion. The fibres are thick, and arise either from the cranial pole of the inferior colliculus, the caudal part of the superior colliculus, the nucleus parabigeminus, or several of these structures. The most cranial region only of the inferior colliculus possibly contributes to this fibre pathway. The fibres are distributed among those of the lateral fillet (Text-fig. 1), and terminate in the lateral pontine nucleus of the same side.

It is probable that a few fibres extend from the inferior into the three deeper layers of the superior colliculus. The injury to the dorsal nucleus of the lateral lemniscus has led to degeneration in the dorsal commissure of the lateral fillet or commissure of Probst (Pl. II, fig. 2). This degeneration is more than that occurring in this commissure in Exp. 1. The course and terminations

of the commissure show the same features in Exp. 4, and are described under that heading.

Text-fig. 1. Exp. 2. Photomicrograph of a transverse section showing degenerate tecto-pontine fibres among the fibres of the lateral fillet. Chlorate-osmic-formalin. ×60.

Experiment 3

In a guinea-pig a lesion was placed in the right inferior colliculus. The post-operative course was uneventful.

Histological examination

Lesion. A wedge-shaped portion of tissue has been cut out of the dorso-lateral aspect of the caudal two-thirds of the right inferior colliculus. Caudally the lesion affects the dorso-lateral two-thirds of the colliculus, while cranially it is confined to the surface.

Fibre degeneration. There is much degeneration in the right inferior brachium, the fibres ending in the caudo-ventral nucleus of the medial geniculate body. The inferior intercollicular commissure contains many degenerate fibres, which end in the dorsal half of the uninjured inferior colliculus.

Experiment 4

In a guinea-pig a lesion was made in the ventral region of the right inferior colliculus. The animal made an uneventful recovery.

Histological examination

Lesion. The lesion affects the ventral region of the caudal two-thirds of the right inferior colliculus, the dorsal nucleus of the lateral lemniscus, and the lateral part of the brachium conjunctivum and dorsal nucleus of the brachium conjunctivum. The caudal part of the right inferior brachium has been cut across.

Fibre degeneration. There are many degenerate fibres in the right inferior brachium, ending in the caudo-ventral nucleus of the medial geniculate body. Many degenerate fibres course in the inferior intercollicular commissure, and end in the dorsal half of the inferior colliculus of the opposite side.

No statement can be made as to whether fibres from the region of the lesion reach the homolateral mesencephalic nucleus or central grey matter, because of pseudo-Marchi reaction in the proximity of the injury.

The dorsal nucleus of the lateral fillet—and possibly also the ventro-lateral region of the inferior colliculus—gives rise to fibres of the commissure of Probst, which shows more degeneration than in Exp. 1. The fibres of the commissure course medialwards, lying ventral to the dorsal nucleus of the brachium conjunctivum, and then through the ventral region of the central grey matter and the homolateral nucleus of the trochlear nerve (Pl. I, fig. 2). It is improbable that any of the fibres end in the latter nucleus. The fibres turn ventralwards and pass through the ipsilateral fasciculi confines and medial longitudinal bundle, to which they do not contribute. At the midline the fibres lie immediately ventral to the medial longitudinal bundles (Pl. I, fig. 2). The fibres continue towards the opposite side, lying ventral to the heterolateral medial longitudinal bundle and fasciculi confines. Some end in the contralateral dorsal nucleus of the brachium conjunctivum and dorsal nucleus of the lateral lemniscus. Others turn dorsalwards and, running with fibres of the lateral fillet, end in the anterior part of the central nucleus of the heterolateral inferior colliculus. The commissural fibres are disposed somewhat obliquely, occupying a slightly more cranial plane at their terminations than at their origin.

Many lateral and ventral fibres of the right brachium conjunctivum have degenerated. They reach the contralateral red nucleus, in which many end. Others, on reaching this position, turn dorsalwards, and penetrating among the fibres of the medial longitudinal bundle and medial fasciculi confines, terminate in the oculomotor nucleus on the side opposite to that upon which the lesion was inflicted. A few degenerate fibres of the brachium conjunctivum continue cranially beyond the red nucleus, and turn dorsally or dorso-laterally to end in the thalamus. They are too few to determine the nucleus in which they end.

Experiments 5 and 6

In two cats in which electrolytic lesions were placed in the cranio-ventral region of the midbrain, the needle electrode passed through the dorsal nucleus of the lateral fillet, which was thus incidentally injured. The post-operative symptoms were referable to the main lesions, and do not call for comment in the present text.

Histological examination

Lesions. In each case the medial half of the left dorsal nucleus of the lateral lemniscus and adjoining lateral fillet fibres have been destroyed. The main lesions and the fibre degeneration ensuing from them are not discussed.

Fibre degeneration. In both cases degenerate fibres, from the lesion, run dorsalwards with the lateral fillet. Most of them end in the homolateral inferior colliculus, mainly in the central nucleus. A few course along the medial aspect of the inferior colliculus and cross in the inferior intercollicular commissure, ending in the contralateral inferior colliculus.

There is much degeneration in the commissure of Probst. In the cat the fibres of this system are disposed in a strictly transverse plane; otherwise they have a similar course and termination as in the guinea-pig.

Experiment 7

In a cat an electrolytic lesion was placed in the left inferior colliculus. The animal made an uninterrupted recovery.

Histological examination

Lesion. The dorsal third of the caudal third of the left inferior colliculus has been destroyed. More anteriorly the lesion is limited to the dorso-medial fourth of the inferior colliculus. At the level of the cranial third of the inferior colliculus the lesion also extends into the dorso-lateral region of the central grey matter, almost reaching the ependyma of the aqueduct. Cranially the lesion involves the dorso-medial surface of the caudal region of the superior colliculus.

Fibre degeneration. Degenerate fibres course from the lesion across the uninjured part of the inferior colliculus, and reach the homolateral inferior brachium, to the lateral region of which they are confined. There are many degenerate fibres in the inferior intercollicular commissure. Some of them end in the dorsal half of the inferior colliculus of the opposite side, while others enter the heterolateral inferior brachium. In this they attain a lateral position. The degenerate fibres in the inferior brachia terminate in the caudo-ventral nuclei of the corresponding medial geniculate bodies.

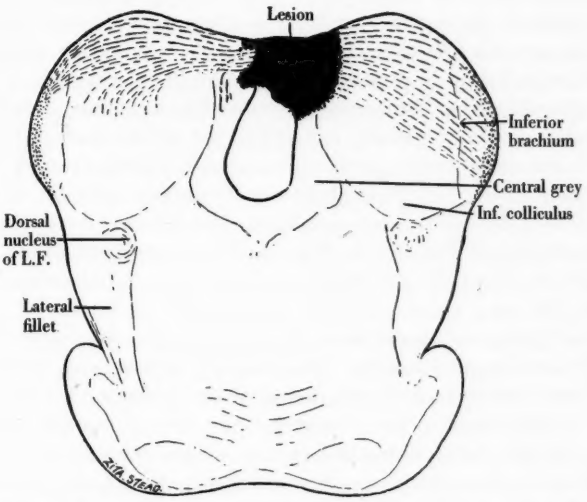
Degenerate fibres occur in the crossed and uncrossed tecto-spinal tracts. They arise from the injured portion of the superior colliculus. From this region tecto-pontine fibres also arise. They form a bundle external to the lateral fillet, ending in the lateral pontine nucleus of the same side.

Experiment 8

In a cat an electrolytic lesion was made in an inferior colliculus. The animal recovered uneventfully.

Histological examination

Lesion. **The lesion affects the dorso-medial region of the caudal two-thirds of the inferior colliculus. Caudally, the dorso-medial sixth of the colliculus has been destroyed. Cranially the lesion is smaller, and involves the junction of the inferior colliculus with the inferior intercollicular commissure. The cranial half of the lesion includes the dorsal region of the central grey matter, and extends into the aqueduct (Text-fig. 2).**



Text-fig. 2. Exp. 8. Drawing of a transverse section at the level of the middle of the inferior colliculi, showing the lesion and degenerate fibres.

Fibre degeneration **(Text-fig. 2). From the site of the lesion many degenerate fibres course across the uninjured part of the inferior colliculus and enter the ipsilateral inferior brachium. They are confined to the lateral half of the brachium, and end in the caudo-ventral nucleus of the medial geniculate body.**

There are many degenerate fibres in the inferior intercollicular commissure. Some of them end in the dorsal half of the contralateral inferior colliculus. The majority enter the heterolateral inferior brachium—in which they occupy the lateral region—and end in the caudo-ventral nucleus of the medial geniculate body.

There are some degenerate, irregularly disposed, short fibres in the central grey matter.

DISCUSSION OF RESULTS

The distribution of the lateral fillet. The course and distribution in the medulla oblongata of the fibres arising from the ventral and dorsal cochlear nuclei and the vestibular nerve as established in Exp. 1 are in agreement with the observations of Held (1891, 1892, 1893), Tschermak (1899), Cajal (1909–11), Lewy (1909), Yoshida (1924, 1925) and others on Weigert, Golgi and Marchi preparations of various mammals. There is some discrepancy regarding the terminations of the lateral fillet in the midbrain, medial geniculate body and possibly cerebral cortex. Held, from Weigert and Golgi preparations of brains of the cat, rabbit, chimpanzee and human foetus, concludes that fibres from the lateral fillet end in the homolateral dorsal nucleus of the lateral fillet, homolateral and contralateral inferior and superior colliculi, and cerebral cortex. Fibres from the cochlear nerve and the secondary auditory relay nuclei are supposed to reach the cortex. Most of the fibres to the cortex are stated to course in the superior brachium. The medial geniculate body is not mentioned. The auditory radiation is considered to consist of lateral fillet fibres and a smaller contribution from the inferior colliculus. Van Gehuchten (1906*a*, *b*), from Marchi studies on the guinea-pig, concludes that all the fibres of the cochlear nerve end in the dorsal and ventral cochlear nuclei. Kölliker (1896), from Golgi preparations of the cat and Weigert material of the human brain, describes the lateral fillet as ending in the dorsal nucleus of the lateral fillet and the inferior and superior colliculi. Tschermak, from Marchi preparations of the brain of a cat in which the trapezoid body had been sectioned, concludes that fibres from the lateral fillet end in the inferior and superior colliculi. The homolateral fibres of the lateral fillet arise mainly from the superior and accessory olivary, medial praeolivary and trapezoid nuclei. This is in agreement with the findings of Held and Cajal, who also, however, include in the ipsilateral lateral fillet fibres from the cochlear nuclei. The occurrence of a few degenerate fibres in the lateral fillet of the operated side in Exp. 1 is in agreement with the interpretations of Held, Tschermak and Cajal. Cajal (1902), from Golgi preparations of the brain of the mouse, describes the majority of the fibres of the lateral fillet as entering the inferior brachium and ending in the medial geniculate body. Many of the fibres send collaterals into the inferior and superior colliculi, in which also terminate a few direct fibres. Some fibres of the lateral fillet cross in the inferior intercollicular commissure to end in the inferior colliculus of the opposite side. Valeton (1907), from myelogenesis studies on human material, concludes that fibres of the lateral fillet end in the inferior and superior colliculi. Valeton (1908) also states, from observations on Weigert preparations of a large series of mammalian brains, that some fibres of the lateral fillet cross in the anterior medullary velum to end in the inferior colliculus of the opposite side. No degenerate fibres have been observed in the anterior medullary velum in any of the present experiments involving the lateral fillet (Exps. 1, 5 and 6), dorsal nucleus of the lateral fillet (Exps. 2, 4, 5 and 6) and inferior colliculus (Exps. 2, 3, 4, 7 and 8). Lewy, from Marchi studies on the cat and rabbit,

describes the lateral fillet as ending in the inferior colliculus. Castaldi (1926), from investigation of the brains of embryonic and neonatal guinea-pigs prepared by the methods of Cajal and Weigert, concludes that the superior colliculus receives a small number of lateral fillet fibres.

Exp. 1 shows that in the guinea-pig the majority of the lateral fillet fibres arising from the dorsal and ventral cochlear nuclei end in the contralateral inferior colliculus. A small number of fibres ends in the contralateral ventral and dorsal nuclei of the lateral lemniscus. A few fibres end in the medial geniculate body, while the termination of lateral fillet fibres in the superior colliculus is doubtful; if any are present they are few in number. In Exp. 1 the possibility of fibres from the superior olivary nuclear complex or the trapezoid nuclei reaching the superior colliculus is not excluded. It is unlikely, however, that any appreciable proportion of lateral fillet fibres end in the superior colliculus, since in Exps. 4, 5 and 6 no fibres were seen to extend from the region of the lesion into this structure, whilst in Exp. 2 only a few fibres possibly course from the injured area into the superior colliculus. In each of these cases the lesion should have interrupted some of the lateral fillet fibres that may go to the superior colliculus.

A small group of fibres arising from the dorsal and ventral cochlear nuclei terminates in the homolateral nuclei of the lateral fillet and inferior colliculus.

The inferior brachium. The contribution of the lateral fillet to the inferior brachium has been discussed in the preceding section. The majority of the fibres in the inferior brachium are derived from the inferior colliculus of the same side. Valeton (1908) and Obersteiner (1912), the latter from Weigert preparations of the human brain, claim that fibres from the inferior intercollicular commissure enter the inferior brachium. Jelénska-Macieszyna (1911), from Marchi material of the rabbit, denies the existence of such fibres. Similar fibres have been observed in Exps. 7 and 8 on cats. In these cases the lesions involve the dorso-medial region of the inferior colliculus. No fibres from the inferior intercollicular commissure have been observed to enter the inferior brachium in Exps. 2, 3 and 4 on guinea-pigs, in which the lesions in the inferior colliculus have not affected the dorso-medial region of this structure.

Jelénska-Macieszyna states that, while possibly a few fibres of the inferior brachium end in the "dorsal" nucleus of the medial geniculate body, the majority end in the "ventral" nucleus. In the present experiments the ascending fibres of the inferior brachium were found to end in the caudo-ventral nucleus of the medial geniculate body.

In Exps. 7 and 8 the degenerate fibres in the homolateral and contralateral inferior brachia are confined to the lateral region of these structures. In these cases the lesions are limited to a small portion of the inferior colliculus, and do not interrupt fibres arising from its uninjured parts. The findings suggest a spatial arrangement of the fibres in the inferior brachium. It is possible that there is a point-to-point relationship between the components of the inferior colliculus and the medial geniculate body. A localization in the medial genicu-

late body with regard to various parts of the audito-sensory cortical area has been shown by Clark (1936*a*) and Walker (1937) in the macaque, from observations on retrograde cell degenerations in the diencephalon after ablation of various portions of the temporal lobe. Poliak (1932) and Woollard & Harpman (1939), from Marchi studies on the macaque and cat respectively, have described a regular arrangement of the geniculo-cortical fibres, so that, for instance, in the cat the fibres from the lateral part of the medial geniculate body reach the caudal, those from the medial part the cranial, those from the dorsal portion the dorsal and those from the ventral part the ventral region of the audito-sensory cortex. Ades *et al.* (1939), by the method of conditioned reflexes, have shown that in the cat there is a precise spatial localization of different sound frequencies in the medial geniculate body. Poliak (1927), Lorente de Nó (1933) and Lewy & Kobrak (1936) have demonstrated a regular pattern in the projection of the organ of Corti on the cochlear nuclei. Culler (1935), Stevens *et al.* (1935) and Culler *et al.* (1937) have shown that the several segments of the cochlea respond differentially to acoustic stimuli of different frequencies. There is thus a precise localization of function in the auditory system. A point-to-point projection extending from the organ of Corti to the cerebral cortex is the requisite of the Helmholtz theory of hearing or its modifications. Such a relationship has been shown to exist between the medial geniculate body and the cortex, and the organ of Corti and the cochlear nuclei; the observations of Exps. 7 and 8 suggest that it may also occur between the inferior colliculus and the medial geniculate body. The observations of Lewy indicate at least an orderly disposition of the fibre connexions of the medullary auditory nuclei.

Winkler (1921) states that fibres from the inferior colliculus reach the cortex via the inferior brachium. In the present experiments no ascending fibres in the posterior brachium have been observed to reach any structure other than the caudo-ventral nucleus of the medial geniculate body.

Descending fibres from the inferior colliculus. There is much controversy on the contribution that the inferior colliculi may make to descending tectal connexions. Held describes fibres from the inferior colliculus and dorsal nucleus of the lateral lemniscus coursing among the fibres of the lateral fillet and ending in the homolateral superior olivary and trapezoid nuclei. Fibres from the inferior colliculus to the dorsal nucleus of the lateral fillet are also described. Kölliker denies the existence of any of these fibres. Cajal describes crossed and uncrossed descending pathways arising from the anterior region of the inferior colliculus; the crossed fibres form part of the tecto-spinal tract. Each pathway consists of fibres from both inferior colliculi, those derived from the contralateral inferior colliculus crossing in the inferior intercollicular commissure. The destination of these tracts is unknown. Winkler (1920) mentions fibres from the inferior colliculus to the dorsal nucleus of the lateral fillet. Castaldi (1926) describes homolateral tecto-pontine fibres arising from cells along the lateral aspect of the inferior colliculus, and "recurrent tecto-cochlear fibres of the lateral fillet" which, arising from the "principal" nucleus

of the inferior colliculus, descend in the lateral fillet. The terminations of these tracts are not specified. Tsai (1925), from Weigert preparations of the brain of the opossum, describes various fibre systems from the superior and inferior colliculi to the lateral nucleus of the pons and the substantia nigra. The tracts described are stated to arise principally from the inferior colliculi. Rioch (1929), from similar material of carnivores, describes tecto-nigral and nigro-tectal tracts associated mainly with the inferior collicular portion of the mesencephalic tectum.

Observations using the Marchi technique have also led to conflicting results. Tricomi-Allegra (1907), in the rabbit, placed lesions in the anterior part of the inferior colliculus. Degenerate fibres are stated to enter the decussations of Meynert and Forel to reach the heterolateral formatio reticularis and red nucleus. Tecto-pontine fibres arise from the lateral region of the lesions. They lie external to the lateral fillet and end in the pontine nuclei. Jelénska-Macieszyna (1911), in the rabbit, placed lesions in the dorsal half of the inferior colliculus. Descending fibres are described coursing from the caudal region of the central nucleus of the inferior colliculus to the dorsal nucleus of the lateral lemniscus, and possibly also to homolateral vestibular nuclei. Busacca (1921), in the dog, describes an uncrossed set of fibres that, arising from the inferior colliculus, runs at first with the lateral fillet, then lies ventro-lateral to the superior olive, and can be traced as far caudalwards as the second cervical segment of the spinal cord.

The tecto-pontine tract was discovered by Münzer (1898) and Münzer & Wiener (1898, 1902), who named it the superficial tecto-bulbar tract. It was demonstrated by the Marchi method in the pigeon after lesions of the corpus bigeminum, and in the rabbit after lesions of the superior colliculus. In the rabbit the tract arises from the caudal part of the superior colliculus and lies external to the lateral fillet. The majority of the fibres end in the homolateral pontine nuclei. A few fibres reach a somewhat more medial position and end in the neighbourhood of the medial fillet; the figures suggest that they terminate in the trapezoid nuclei. Pawlow (1900) describes the tecto-pontine tract in a rabbit in which a lesion is stated to have been placed in the superior colliculus. Examination of the author's illustrations reveals that the lesion also affected the ventral part of the cranial pole of the inferior colliculus. The fibres were traced in Marchi preparations; they lie external to the lateral fillet and terminate in the homolateral nuclei of the pons. Van Gehuchten (1906*a*, *b*) states that, while from his earlier experiments he believed that Münzer's fibres arise from the inferior colliculus, further work and the examination of Pawlow's specimen have led him to the conclusion that the tecto-pontine tract arises from the superior colliculus. De Lange (1910), in the rabbit, studied descending tectal fibres by the methods of Weigert, Nissl and Marchi. The lesions were made by undercutting the tectum. Crossed and uncrossed tecto-bulbar and tecto-spinal fibres arise from the superior colliculus. Fibres—mostly uncrossed but a few crossed—arise from the caudal part of the superior colliculus and end in

the region of the trapezoid body and pontine nuclei. The cranial extremity of the inferior colliculus may contribute to some or all of the fibre systems described. Jelénska-Macieszyna (1911, 1913), from Marchi studies on the rabbit, concludes that the tecto-pontine tract arises from the caudal region of the superior colliculus. Rasmussen (1936), from Marchi material of the cat, establishes that the tecto-spinal tract arises only from the superior colliculus.

In the present material tecto-pontine fibres were observed in Exps. 2 and 7, in which the lesion includes the caudal extremity of the superior colliculus. In Exp. 2, on a guinea-pig, it is not possible to determine whether the fibres arise from the cranial pole of the inferior colliculus, the caudal part of the superior colliculus, the nucleus parabigeminus, or several of these centres. In Exp. 7, on a cat, degenerate tecto-pontine and crossed and uncrossed tecto-spinal fibres arise from the injured region of the superior colliculus. In the guinea-pig, the more primitive form, the tecto-pontine fibres are scattered among the fibres of the lateral fillet. In the cat they form a compact bundle external to the lateral fillet. The same topographic relation obtains in the rabbit (Münzer & Wiener, Pawlow, Tricomi-Allegra). The tecto-pontine fibres are uncrossed and terminate in the lateral nucleus of the pons. No other descending fibres were observed in the present experiments.

Fibres from the inferior colliculus to the central grey matter. Scattered short fibres from the inferior colliculus to the central grey matter have been described by Cajal and Castaldi. There is no mention in previous literature of the bundle of fibres observed in Exp. 2, arising from the ventro-lateral region of the inferior colliculus and going to the central grey matter and possibly the homolateral mesencephalic nucleus of the fifth nerve.

Fibres from the inferior to the superior colliculus. Whilst Held and Cajal state that cells in the cranial region of the inferior colliculus give rise to fibres ending in the superior colliculus, no mention of such a connexion is made by authors who have applied experimental methods. The results of Exp. 2 indicate that such fibres probably exist, but that they are very few in number. The fibres described by Held from the dorsal nucleus of the lateral fillet to the superior colliculus have not been observed in the present material.

Negative results. Cajal describes fibres from the inferior colliculus contributing to the commissure of Gudden. Castaldi traced this commissure in two guinea-pigs in which the optic tracts were atrophied by excising the eyes. The commissure could be traced no further caudalwards than the medial geniculate bodies. Weaver (1937), from experimental studies on the cat, emphasizes that the commissures of Meynert and Gudden are one complex, the inferior hypothalamic decussation. While Gudden (1879) believed it to connect the medial geniculate bodies, there is as yet no satisfactory evidence of its origin, which would appear to be several diencephalic nuclei. In the present experiments no degenerate fibres were observed in the inferior hypothalamic decussation.

No fibres from the inferior colliculus were found to join the tecto-cerebellar

tract or to reach the cerebellum by any other way. This observation is in accord with the results of previous workers.

Fibres from the dorsal nucleus of the lateral lemniscus. Kölliker describes fibres coursing from the dorsal nucleus of the lateral fillet into the homolateral inferior colliculus, and remarks that possibly fibres from the dorsal nucleus of the lateral fillet enter the inferior intercollicular commissure. A few degenerate fibres reaching the inferior colliculus of the opposite side through the inferior intercollicular commissure from lesions involving the dorsal nucleus of the lateral fillet and adjoining lateral fillet fibres were observed in Exps. 5 and 6 on cats, but not in Exps. 2 and 4 on guinea-pigs. Degenerate fibres enter the homolateral inferior colliculus in Exps. 5 and 6, but it is not certain whether they are derived from the dorsal nucleus of the lateral lemniscus or whether they are lateral fillet fibres.

The fibres of the commissure of Probst (dorsal commissure or transverse fibres of the lateral fillet) are considered to be derived from four sources: collaterals of fibres of the lateral fillet (Held, Cajal, Castaldi), the dorsal nucleus of the lateral lemniscus (Held, Castaldi), the inferior colliculus (Held, Kölliker) and the nucleus of Kölliker-Fuse (Castaldi). The first two sources are confirmed by Exps. 1, 2, 4, 5 and 6 of the present series. No fibres have been traced into the commissure in Exps. 3, 7 and 8, in which the lesions are confined to the dorsal part of the inferior colliculus. Whether fibres from the ventral part of the inferior colliculus or from the nucleus of Kölliker-Fuse take part in the commissure has not been determined in the present experiments. The course of these fibres in the guinea-pig, described in the record of Exp. 4, agrees with the observations of Castaldi on the same animal. The following are some of the terminations of the fibres of the commissure of Probst that have been inferred from the study of normal material: the contralateral inferior colliculus (Held), the homolateral nucleus of the trochlear nerve (Ziehen, 1903*a*, *b*), the contralateral reticular nuclei and the mesencephalic nucleus (Bechterew & Lagursky, 1897), the contralateral dorsal nucleus of the lateral fillet (Lewandowsky) and the medial longitudinal bundle (Winkler, 1920). It may form a contralateral descending tract (Castaldi). The present observations show that the fibres of this system end in the contralateral dorsal nucleus of the lateral fillet, dorsal nucleus of the brachium conjunctivum and central nucleus of the inferior colliculus. The dorsal nucleus of the brachium conjunctivum is the dorsolateral of two cell masses depicted by Winkler & Potter (1911, 1914) lying in relation to the superior peduncle, and especially well developed at the level of the inferior colliculus.

Cajal and Huber & Crosby (1929) describe fibres from the dorsal nucleus of the lateral fillet to the medial geniculate body of the same side. Such fibres have not been observed in Exps. 5 and 6, in which the lesions were limited to the dorsal nucleus of the lateral fillet and adjacent lateral fillet fibres.

Incidental findings. The distribution of degenerate fibres in the cerebellum observed in Exp. 1 is in agreement with the generally accepted cerebellar

terminations of the vestibular nerve, restiform body and dorsal spino-cerebellar tract.

In Exp. 1 fibres were found arising from the region of the lesion and terminating in the homolateral medial vestibular and globose nuclei. They follow a course similar to, but in the reverse direction of, the fasciculus uncinatus of Russell. They are possibly those described by Lewy as the radix verticalis, connecting the ganglion of Scarpa with the superior and medial vestibular nuclei. The radix verticalis runs like the fasciculus uncinatus, lying dorsal to the restiform body after passing through the ventral cochlear nucleus.

The termination of fibres of the brachium conjunctivum in the contralateral oculomotor nucleus observed in Exp. 4 has been described by Wallenberg (1898), Klimoff (1901), Saito (1922, 1923), Riese (1925) and Clark (1936*b*).

CORRELATION OF THE ANATOMICAL FINDINGS WITH PHYSIOLOGICAL WORK

Electrical stimulation of the inferior colliculus has given discrepant results. Ferrier (1876), Prus (1899) and Bechterew (1909), working on the monkey, dog, cat, rabbit, guinea-pig, rat and sheep, obtained a variety of responses, including clonic and tonic extensor spasms, walking movements, deviation of the head, body and eyes to the opposite side, and of the head and body to the same side when stronger stimuli were employed, rotation, erection and abduction of the ears (especially that of the opposite side), dilatation of the pupil, slowing of respiration and pulse rates, and rise in blood-pressure. Prus obtained in the dog the same motor reactions of the head, body and eyes on painting the inferior colliculi with cocaine. Recent workers, on electric stimulation of the inferior colliculus in the cat, record no effect on the pupil (Ingram *et al.* 1931), blood pressure (Kabat *et al.* 1935), muscle tone of the bladder (Kabat, 1936) or respiration (Kabat *et al.* 1936). These investigators employed currents of moderate intensity and accurately localized the point of stimulation by the Clarke-Horsley stereotaxic instrument. Some of the fibre pathways concerned in the control of blood pressure, muscle tone of the bladder and rate and amplitude of respiration lie close to the medial border of the inferior colliculus. It is probable that at least some of the reactions obtained by earlier investigators were due to spread of the electric current to neighbouring fibres or nuclei.

Ferrier, Marckwald, Lewandowsky (1896), Prus and Bechterew, from ablation and electric stimulation experiments, conclude that the inferior colliculus influences respiration and phonation. Similar experiments by Klemperer, Grabower (1897, 1911), Spiegel & Enghoff (1925), Spiegel & Ullmann (1926) and Kabat *et al.* (1936) offer no support to the conception that the inferior colliculus is related to the mechanism of respiration or phonation.

Unilateral removal of an inferior colliculus in the rat, rabbit, and guinea-pig leads, according to Bechterew (1895, 1909), to transient absence or diminution of reflex movements of the contralateral external ear to auditory stimuli. Spiegel & Kakeshita (1926), working on the cat and guinea-pig, found that

removal of the mesencephalic tectum or section of the brain stem caudal to the inferior colliculi does not abolish reflex movements of the auricular muscles, closing of the eyelids or contraction of the body musculature to auditory stimuli, provided these are sufficiently loud. Forbes & Sherrington (1914), after section of the brain stem cranial to the inferior colliculi, obtained similar motor reactions employing auditory stimuli of moderate intensity. Spiegel & Kakeshita conclude that the inferior colliculi may be reflex centres for "certain kinds" of auditory stimuli. Motor responses to sounds may be subserved by collaterals from the trapezoid body to the facial nucleus and reticular nuclei of the hindbrain (Held, 1893), and by fibres from the superior olive to the medial longitudinal bundle and the nucleus of the abducens nerve (Yoshida, 1925).

The present observations disclose three possible fibre pathways for reflex activities of the midbrain auditory centres. They are: the fibres from the ventro-lateral region of the inferior colliculus to the central grey matter and possibly the mesencephalic nucleus of the fifth nerve, the fibres of the commissure of Probst terminating in the dorsal nucleus of the brachium conjunctivum, and the probably existing fibres from the inferior to the superior colliculus. The significance of the first fibre connexion is doubtful. Kappers *et al.* (1936) state that the nuclei of the brachium conjunctivum contribute fibres to the superior peduncle and probably receive fibres from the ventral spino-cerebellar tract. They are, therefore, probably motor centres. Gerver has shown that a much stronger electric current is required to obtain movements of the eyes by electric stimulation of the inferior than of the superior colliculus. Stimulation of the inferior colliculi does not produce movements of the eyes when they have been separated from the superior colliculi by a cover-slip. The paucity of the possible reflex pathways from the inferior colliculus indicates that the majority of the motor reactions obtained by electric stimulation of this structure are due to diffusion of the current.

Marchi studies by the present writers have shown that in the cat and guinea-pig fibres arising from the temporal region of the cerebral cortex end in the homolateral medial geniculate body and inferior colliculus. In the cat the fibres to the medial geniculate body arise from the audito-sensory area. These fibres are also described by Mettler (1932), from similar material. The exact origin of the cortical fibres to the inferior colliculus in the cat remains to be determined. In the macaque the medial geniculate body receives fibres from the superior temporal gyrus, and the inferior colliculus is supplied with fibres from most of the temporal region (Mettler, 1935). Woollard & Harpman have shown that the medial geniculate body gives rise to no fibre tracts other than the auditory radiation and possibly Gudden's commissure. It is, therefore, a purely sensory centre. The observations recorded in the present paper show that the inferior colliculus is almost wholly a sensory nucleus. It is, therefore, postulated that the fibres from the temporal region of the cortex to the medial geniculate body and inferior colliculus exert a controlling effect upon the sensory impulses received by these centres. Brouwer (1928) has similarly

suggested that the fibres extending from the visual cortical area to the lateral geniculate body transmit inhibitory impulses.

SUMMARY

1. The connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus have been studied in four guinea-pigs and four cats by the method of Marchi and its modification by Swank and Davenport.

2. The majority of the lateral fillet fibres arising from the cochlear nuclei end in the contralateral nuclei of the lateral fillet and central nucleus of the inferior colliculus.

3. Some fibres from the cochlear nuclei end in the homolateral nuclei of the lateral fillet and inferior colliculus.

4. A few lateral fillet fibres end in the medial geniculate body.

5. The termination of lateral fillet fibres in the superior colliculus is problematical.

6. In the guinea-pig, the inferior brachium contains fibres derived from the homolateral inferior colliculus and a few lateral fillet fibres; in the cat it also receives fibres from the contralateral inferior colliculus through the inferior intercollicular commissure.

7. The ascending fibres in the inferior brachium end in the caudo-ventral nucleus of the medial geniculate body.

8. In two cats the findings suggest a spatial arrangement of the fibres in the inferior brachium.

9. Tecto-pontine fibres arise, in the guinea-pig, from either the cranial pole of the inferior colliculus, the caudal part of the superior colliculus, the nucleus parabigeminus, or several of these centres. They course among the fibres of the lateral fillet, and end in the ipsilateral lateral pontine nucleus.

10. Tecto-pontine fibres arise, in the cat, from the caudal region of the superior colliculus. They form a bundle external to the lateral fillet, and terminate as in the guinea-pig.

11. Fibres arise from the ventro-lateral region of the inferior colliculus, course medialwards, and end in the central grey matter and possibly in the homolateral mesencephalic nucleus.

12. A few fibres probably run from the inferior to the superior colliculus.

13. The commissure of Probst, or dorsal commissure of the lateral fillet, receives fibres from the dorsal nucleus of the lateral lemniscus and collaterals from lateral fillet fibres. The fibres of the commissure end in the contralateral dorsal nucleus of the lateral lemniscus, dorsal nucleus of the brachium conjunctivum, and central nucleus of the inferior colliculus.

14. Other fibres stated in the literature to arise from the inferior colliculus and dorsal nucleus of the lateral fillet have not been found.

15. The anatomical findings are correlated with physiological work. It is concluded that the midbrain auditory nuclei subserve very few reflex activities, being almost wholly sensory centres.

16. It is suggested that the fibres from the temporal region of the cortex to the medial geniculate body and inferior colliculus control the sensory impulses received by these centres.

Our thanks are due to Mr F. J. F. Barrington for performing the operations in Exps. 7 and 8.

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EXPLANATION OF PLATES I AND II

PLATE I

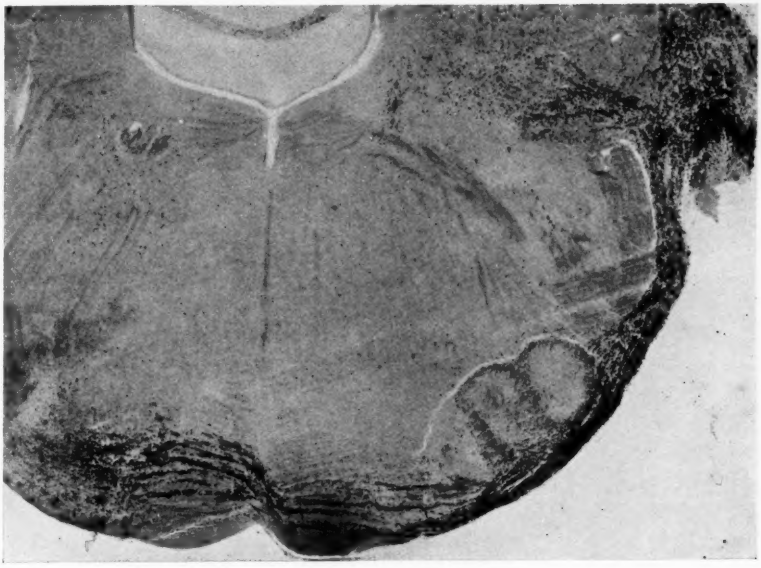
Fig. 1. Exp. 1. Photomicrograph of a transverse section at the level of the cochlear nuclei, showing the lesion and the degeneration in the fibres of the vestibular nerve, trapezoid body and acoustic stria. Marchi. ×20.

Fig. 2. Exp. 4. Photomicrograph of a transverse section at the level of the junction of the caudal with the middle third of the inferior colliculi. The figure shows the relation of the degenerate fibres of the commissure of Probst to the fasciculi confines, medial longitudinal bundles and homolateral nucleus of the trochlear nerve. Marchi. ×60.

PLATE II

Fig. 1. Exp. 1. Photomicrograph of a transverse section at the level of the middle of the inferior colliculi, showing the termination of degenerate fibres of the lateral fillet in the dorsal nucleus of the lateral fillet and central nucleus of the inferior colliculus. Marchi. ×50.

Fig. 2. Exp. 2. Photomicrograph of a transverse section at the level of the middle of the inferior colliculi. The figure shows the lesion, a bundle of fibres arising from the ventro-lateral region of the colliculus and ending in the central grey matter and possibly the homolateral mesencephalic nucleus, and degeneration in the commissure of Probst. Chlorateosmic-formalin. ×27.



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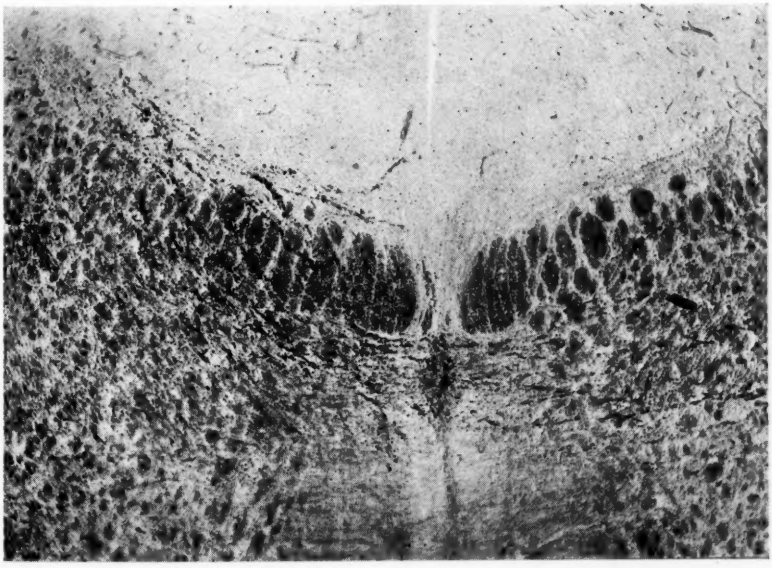

Fig. 2.

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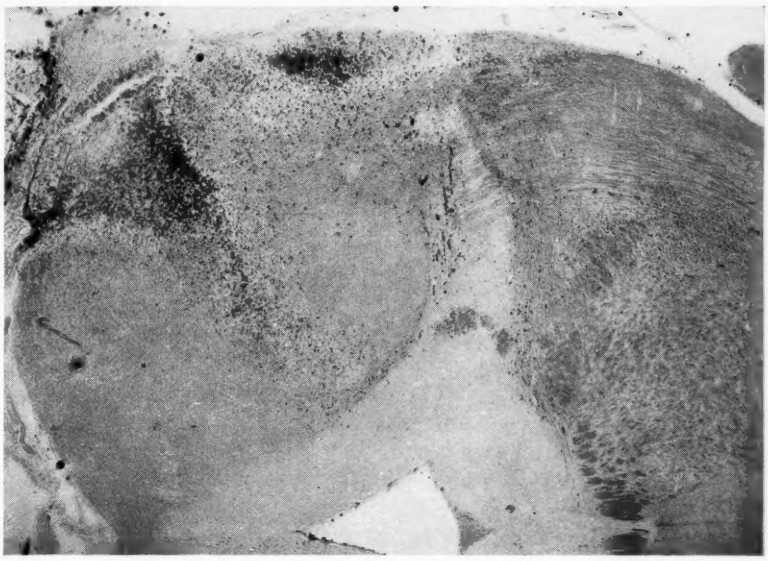

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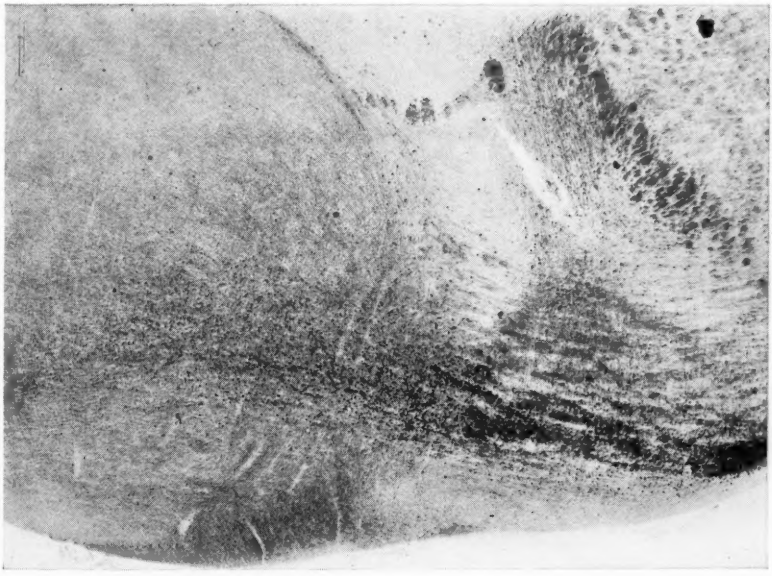

Fig. 1.

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DIFFERENTIATION OF PITUICYTES IN THE HUMAN FOETUS

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By using silver impregnation stains Retzius (1894), Berkley (1894), Herring (1908), and Tello (1912) showed that the parenchymatous cells of the pars nervosa of the pituitary were neuroglial in nature. More recently, Bucy (1930) published a detailed account of these cells in ox material and called them pituicytes. Gersh (1939) described the granules in these cells in the embryonic and adult material of a number of animals.

This study is based on sections of seventeen pituitaries of human foetuses ranging in age from 3 months to the new-born. Shortly after abortion each foetus was placed in refrigeration until the time of autopsy. Some of these remained in the refrigerator as long as 60 hr. without making any apparent difference in the staining of the pituicytes.

Fixation was effected by the Cajal formalin-ammonium-bromide method as described by Penfield & Cone (1937). The time of fixation ranged from 2 days to 3 weeks at room temperature, after which the tissue was washed and sectioned on the freezing microtome. The Hortega silver carbonate methods were employed, either for oligodendroglia or for microglia, as described by Penfield & Cone. The writer, like Bucy, found the silver impregnation methods that stained astrocytes were ineffective as pituicyte stains.

Three-month foetus. The ages of the two youngest foetuses studied were estimated by the internist at 2 months, but measurements suggest the age was nearer to 3 months. Judging by the general appearance of these pituitaries they are comparable to those shown by Tilney (1936) in his Fig. 19 representing the 55 mm. human embryo. In these 3-month embryos the pars nervosa is triangular in shape with the base against the pars intermedia (Pl. I, fig. 3). The outer zone of the nervosa contains many fibres and practically no cells. The nature and origin of the fibres were not determined, but they are not processes of the pituicytes.

The central area of the pars nervosa at 3 months is filled with densely packed nuclei having very little cytoplasm. They vary in shape from round to oval and are directed along the long axis of the section. The round nuclei vary in size from 6 to 10 μ, and the more elongated oval ones from 5 μ wide and 11 μ long, up to others 8 μ wide and 14 μ long. The nuclear membrane is thick and clearly seen. The darkly stained chromatin is present in granular form, there being in each nucleus from twenty to forty of these granules suspended in a distinct linin network. Nucleoli were not identified.

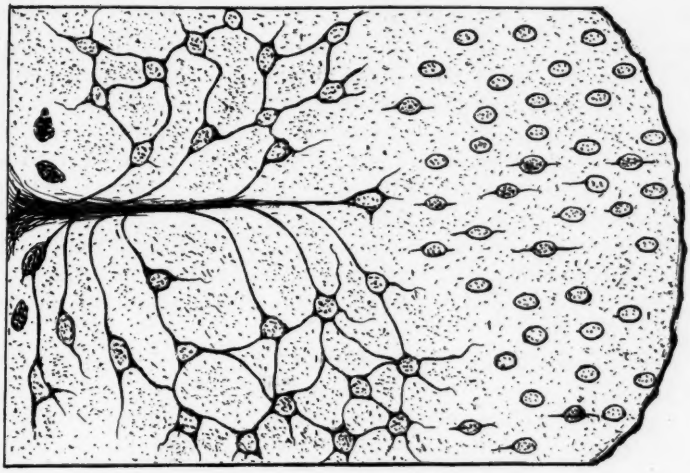
Four-month foetus. During the fourth month those cells in the pars nervosa nearest to the pars intermedia undergo a series of changes whereby more cytoplasm accumulates around the nuclei, and processes make their appearance. By the middle of the fifth month the pituicytes in that part of the pars nervosa proximal to the pars intermedia are well developed, whereas those pituicytes in the distal half are still in the earliest stages of differentiation (Text-fig. 1; Pl. I, fig. 4; Pl. II).

Five-and-a-half-month foetus. The following is a description based on sections of a 5½-month foetus. The part distal to the pars intermedia will be described first. The most striking thing observed is the similarity of the pituicytes in this portion to those observed in the 3-month foetus. The two most conspicuous elements are numerous large nuclei and abundant intercellular granules (Pl. II, fig. 6). Although the nuclei are similar to those described for the 3-month stage, many more are now oval to fusiform in shape. In general, the round nuclei are more lightly stained and the nuclear contents are clearly seen, whereas the oval nuclei are stained more darkly and the nuclear contents are mostly obscured. The more darkly stained nuclei have more abundant cytoplasm, and frequently one, or at times two, processes extending from their ends. These processes are nearly always directed towards the pars intermedia or towards the connective tissue capsule covering the outside of the pars nervosa. At this stage these rudimentary processes are unbranched. Although abundant small reddish brown intercellular granules are seen in this portion, intracellular granules are not present.

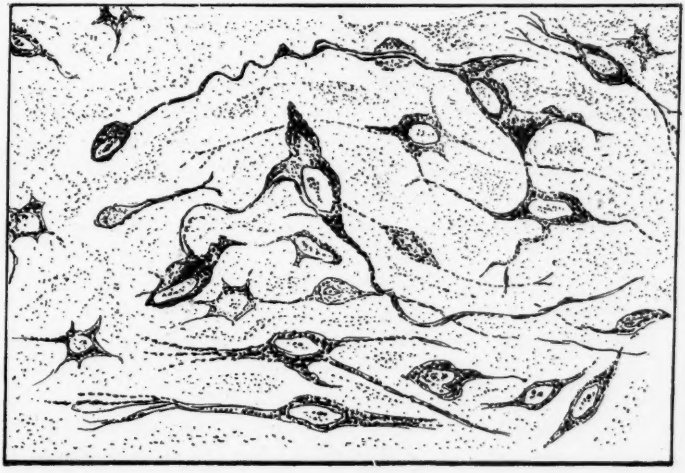
The photomicrographs of the area proximal to the pars intermedia (Pl. I, fig. 4; Pl. II, fig. 7) show the pituicytes and their processes arranged in a whorl-like formation around a large intruding trabecula of connective tissue. The pituicytes of this area are clearly stained and all are well differentiated. In these cells the nuclear membrane is distinct and more lightly stained than the deeply stained cytoplasm. The nuclei are roughly oval and the cells are stellate in shape. Each cell has from three to six processes. Those pituicytes nearest to the trabecula, or to the walls of blood-vessels, have one of their processes attached to these structures. In some cases the attached process is short and thick, in others it is long and thin. In the older stages these processes are greatly elongated. The remaining processes anastomose with those of the neighbouring pituicytes and form an intricate reticulum (Text-fig. 1; Pl. I, fig. 4; Pl. II, fig. 7).

The spaces between the reticular meshwork are filled with small, closely packed granules. Although about the same size as the granules in the distal undifferentiated part of the pars nervosa, these granules, like the nearby pituicytes, stain a distinct purple, instead of reddish brown like those in the distal portion. The relatively few granules present in the cell bodies and processes of the differentiated pituicytes at this stage increase in size and number in the older stages.

The following description is based chiefly on the pituitary of the new-born



Text-fig. 1. Drawing of pituicytes of the 5½-month human foetus, representing the poorly differentiated and the well differentiated pituicytes to each side of a large connective tissue trabecula.



Text-fig. 2. Drawing of pituicytes of a 7½-month human, premature new-born that lived for 2 months. Pituicytes show anastomosis between processes, granules along one of the processes, also a bulb-like ending on one of the terminals.

with a discussion of the intermediate stages. From the fifth month until the time of birth the proximal area of differentiated pituicytes increases in size at the expense of the distal portion; however, even at the time of birth the pituicytes in about one-fifth of the pars nervosa are still in the relatively undifferentiated state, like those in the distal part of the 5-month foetus.

Seven-month foetus. In the 7-month foetus many more blood-vessels and connective tissue trabeculae are present in the differentiated proximal part of the pars nervosa; furthermore, there is a corresponding increase in the number of pituicytes that have processes attached to the walls of blood-vessels and to the trabeculae. The remaining pituicyte processes still anastomose with one another. The entire field contains more fibres due to the marked increase in length of the pituicyte processes. Intracellular granules at this stage are now much more abundant and are found in the cell body and in many of the processes.

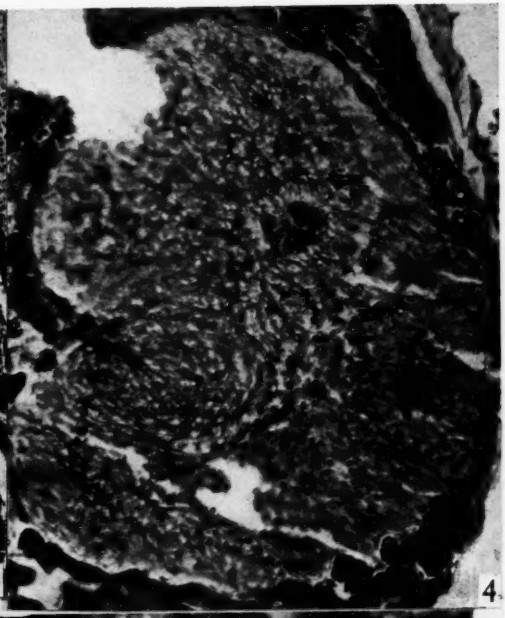
New-born foetus. In the proximal part of the new-born pars nervosa, the trabeculae of connective tissue are now abundant, but there does not appear to be a corresponding increase in the vascularity. It should be noted that there is no similar increase of the connective tissue elements in the nearby poorly differentiated distal area.

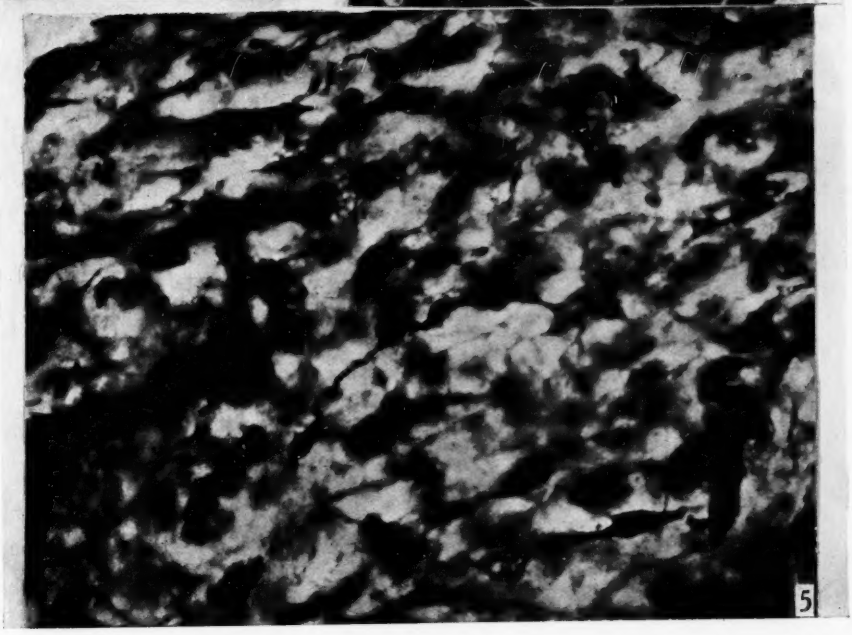
The pituicytes are now considerably larger in size, as are also their nuclei, the cytoplasm, and the intracellular granules. Although many of the pituicyte processes continue to form a rich anastomosis, the processes of others are detached and their cell bodies are no longer stellate-shaped but fusiform. These free processes frequently extend beyond the microscopic field when using the oil-immersion lens. Due to the intensity of the stain very little detail can be observed concerning the finer structure of the processes. Some of them however spread out fan-wise at their free ends, giving the appearance of a brush (Pl. I, fig. 5). In this brush formation the terminal processes of the pituicytes are distinctly fibrillar, with abundant small granules which appear to be attached to the fibrils. Other processes have granules massed about them such as those shown in the photomicrograph (Pl. I, fig. 5) and the drawing (Text-fig. 2). These granules, although clumped in a definite mass, appear to be inside the cell membrane. Other pituicytes are distinctly granular but do not have fibrils.

Some of the pituicyte processes terminate in a peculiar bulb-like ending. The outer part of the bulb is darkly stained, and in the centre there is a small dark body surrounded by clear structureless material. Such a bulb is shown in the photomicrograph (Pl. I, fig. 5) and the drawing (Text-fig. 2). These bulb-like endings may be remnants of pituicyte cell bodies.

At this stage intercellular granules are still present in great profusion, but they are distinctly smaller and more lightly stained than the intracellular granules.

No basophil cells have invaded the pars nervosa from the pars intermedia in any of this foetal material, although many such cells are clearly seen in the adult pituitaries stained by the Hortega silver carbonate method.





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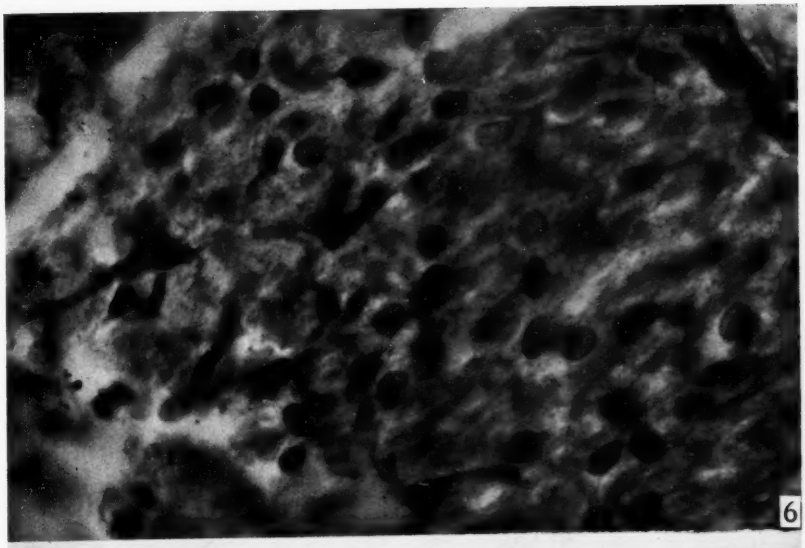

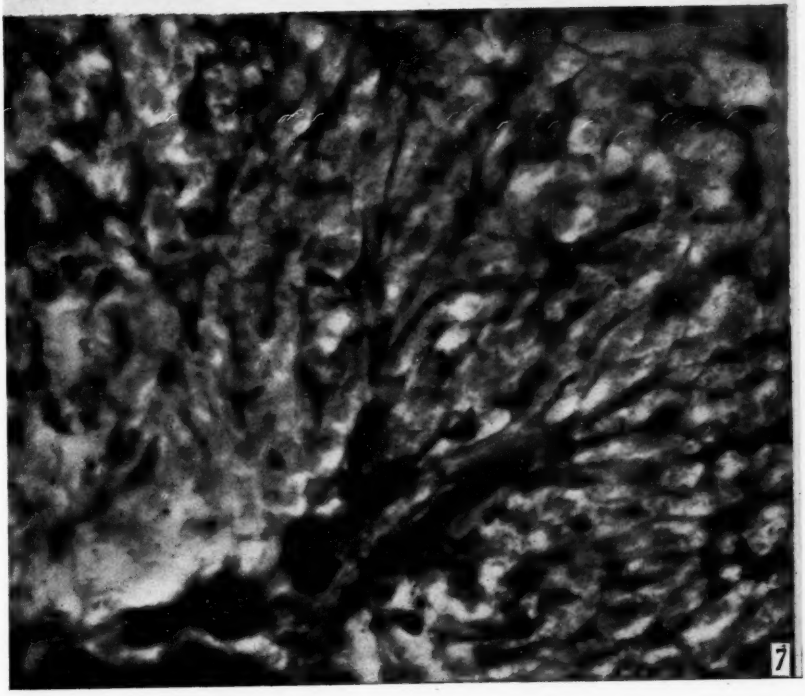

SHANKLIN—Differentiation of pituicytes

UNIV. OF MICH.

SUMMARY

The pituitaries of seventeen human foetuses ranging in age from 3 months to the new-born were studied after staining by the Hortega silver carbonate method.

At 3 months the pituicytes are represented by nuclei that have very little cytoplasm and practically no processes. Following the third month they form three or more processes, some of which become attached to the connective tissue trabeculae or to the walls of blood-vessels, but the majority of the processes join those of nearby pitucytes and form a reticulum.

The most striking observation is the fact that the pituicytes do not all differentiate at the same time. Those near the pars intermedia differentiate first and differentiation proceeds away from this area. At the age of 5½ months all of the pituicytes in the area proximal to the pars intermedia are well developed, but those in the distal part, approximately one-half of the pars nervosa, are similar to those of the 3-month foetus. Even at the time of birth, in about one-fifth of the pars nervosa the pituicytes are still in the primitive state.

The writer is greatly indebted to members of the staff of the Maternity Ward of John Gaston Hospital, Memphis, and of the Pathological Institute, for their co-operation in securing the human material; to Miss Mattie Tharp of the Department of Histology for technical assistance, and to Dr Joseph L. Scianni, Artist of the Pathological Institute for help with the illustrations. All of the above are members of the University of Tennessee Medical School Staff.

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 3. Photomicrograph showing from above downward the pars nervosa, the pars intermedia, cleft, and the pars anterior of a 3-month human foetus. ×95.

Fig. 4. Photomicrograph through the pars nervosa of a 5½-month human foetus showing the differentiated pituicytes in the part proximal to the pars intermedia and the poorly differentiated pituicytes in the distal portion. Note a single clump of blood-vessels deep in the nervosa. ×175.

Fig. 5. Photomicrograph of field corresponding in part to the drawing in Text-fig. 2. Note cells of various shapes, anastomosis, end bulb, and the brush-like ending of one of the pituicyte processes. ×725.

Plate II

Fig. 6. Photomicrograph of same section shown in Pl. I, fig. 4 showing details of cells and intercellular granules in the zone distal to the pars intermedia. ×725.

Fig. 7. Photomicrograph demonstrating details of the proximal area of the same section shown in Pl. I, fig. 4. Note the prominent trabecula, the accompanying blood-vessels stained black, the processes attached to the septum, and processes anastomosing with one another to form a reticulum. Due to lack of focal depth the reticulum is not adequately represented. ×725.

MOUNTING MEDIA AND THE FADING OF HISTOLOGICAL PREPARATIONS

By F. DAVIES

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THERE is a general consensus of opinion that many of the commonly employed histological techniques are somewhat arbitrary or empirical, rather than based upon sound chemical and physical principles. As witness of this may be cited the numerous modifications of most of the well-known techniques that exist, such modifications often not embodying any important chemical or physical variation from the original (apparently unsatisfactory) technique. Nowhere is this lack of sound principle more evident than in the matter of the mounting medium used as a final stage in any technique for the purpose of securing permanency of preparation.

The present communication is concerned with certain chemical and physical features which are desirable in the choice of a suitable mounting medium. It also calls attention to facts, which, in the author's opinion, are not generally appreciated concerning the mounting media commonly used by histologists. Further, experiments have been carried out with various synthetic resins to determine whether any of them are suitable for use as mounting media in microscopy, and it is observed that one such substance appears to have all the important properties which are desirable in a universally employed mounting medium.

OBSERVATIONS

The present work was commenced as a result of the fading of histological preparations which had been stained with iron haematoxylin and Van Gieson's Picro-Säurefuchsin and mounted in commercial "neutral" Canada balsam. In a few days the red-coloured (acid fuchsin) connective tissue had distinctly paled, and in a few weeks the connective tissue was only a faint pink colour. This paling of Van Gieson preparations is well known to occur, and the fading of the red colour is customarily attributed to the Canada balsam becoming *acid*. But it appeared to us that, as in a test-tube Picro-Säurefuchsin is paled and rendered yellow by the addition of an alkali (sodium hydroxide) and the red colour restored by the subsequent addition of an acid (hydrochloric acid), so an acid mounting medium should retain the red stain in the histological preparation and an alkaline mountant cause paling. Further, the addition of an acid to the original Picro-Säurefuchsin did not alter the red colour of the fluid. It was noted that if a fluid, the *p*H of which was 8·0 (ammonia-acetic acid mixture), was added to Picro-Säurefuchsin in a test-tube, the red colour disappeared within ten minutes (the yellow colour of the picric acid remain-

ing). We were thus led to investigate whether the commercial "neutral" Canada balsam which we were using was really neutral (*p*H 7·0) and to extend our investigations to other mounting media.

Now the acidity or alkalinity of a substance can only be assessed in terms of a *p*H if that substance dissolves and undergoes ionization in water (or other ionizing solvent); thus the following two methods were adopted to estimate the *p*H of various mounting media. In the first method a drop of the medium on the end of a glass rod was placed on various B.D.H. paper indicators which had been previously moistened with neutral doubly distilled water, and the resulting colour reaction was noted. The range of paper indicators used was as follows: thymol blue, *p*H 1·2–2·8; bromo-phenol blue, *p*H 2·8–4·6; bromo-cresol green, *p*H 3·6–5·2; bromo-cresol purple, *p*H 5·2–6·8; bromo-thymol blue, *p*H 6·0–7·6; phenol red, *p*H 6·8–8·4; cresol red, *p*H 7·2–8·8; and thymol blue, *p*H 8·0–9·6. In the second method a drop of the mounting medium was shaken up in about ten times its volume of neutral doubly distilled water, and an aqueous indicator (of the above series) subsequently added, the resulting colour reaction being compared with the appropriate series of the B.D.H. capillator. The different volume ratio of medium to water in the second test did not give appreciably different results from those obtained in the paper test. The *p*H thus determined was taken to indicate the *effective* acidity or alkalinity of the medium in so far as any reaction it may undergo with a stain is concerned. The values obtained represent a rough measure of the acidity or alkalinity liable to be produced when the medium comes into contact with water; they would of course be different in other ionizing solvents.

By these methods (and with the reservations indicated) we discovered that our sample of commercial "neutral" Canada balsam had a *p*H of 8·7; that is, it was definitely alkaline, and to this alkalinity we attributed the fading of the preparations. This was confirmed by mounting similarly stained preparations in Canada balsam which we made acid by the addition of benzoic acid, the resulting mixture being found to have a *p*H of 5·6. Such specimens have retained their original depth of red staining over a period (to date) of two years. The specimens mounted at the same time in the commercial "neutral" Canada balsam have now lost entirely their original red colour. Control experiments, kept in the dark, were made concurrently. The results were similar; thus eliminating the possibility of light being a causative agent in the fading process.

An estimation of the *p*H values of various commercial mounting media gave the following results (maker's name expressed in parentheses):

	*p*H
Neutral Canada balsam (Gurr)	4·2
Neutral Canada balsam (Flatters and Garnett)	6·0
Neutral Canada balsam (Nelson)	8·7
Neutral Canada balsam (British Drug Houses)	7·4
Neutral mountant (Gurr)	4·0
Neutral mounting medium (Gurr)	4·0
Neutral balsam (not Canada balsam) (Gurr)	5·5

Natural Canada balsam (Flatters and Garnett)	5·4
Ordinary Canada balsam (Gurr)	4·1
Ordinary Canada balsam (Flatters and Garnett)	3·8
Ordinary Canada balsam (Nelson)	8·6
Ordinary Canada balsam (British Drug Houses)	6·0
Alkaline balsam (Gurr)	8·6
Acid balsam (Gurr)	2·8
Xam (Gurr)	5·4
Euparal (Flatters and Garnett)	3·9
Gum Dammar (prepared by the method of Petersen, quoted by Romeis (1932), which involves removal of impurities by means of animal charcoal)	5·4

It will be seen from the above that there is considerable difference in the *p*H values of the samples of "neutral" media commercially prepared, some being acid and others alkaline, only one of the samples tested approaching neutrality. It may further be remarked that the naturally occurring resinous mixture (Canada "balsam") probably varies in its *p*H value, and that this, combined with the different processes of manufacture of "neutral" balsam, will account, not only for variation in the *p*H values of "neutral" balsams from different makers, but also for variation in the *p*H of "neutral" balsams supplied from the same maker at different times.

Van Gieson preparations mounted in the above media were found to retain their original depth of red stain in the acid media over a period of two years. In the alkaline media, *p*H 8·6 or 8·7, distinct fading was noted in a day or two and the red colour had completely disappeared in six weeks. In the medium of *p*H 7·4, slight fading has occurred over two years. By the addition of an acid (benzoic, salicylic, or phthalic) or of an alkali (nicotine, pyridine, piperidine, diphenylamine, piperazine, quinaldine, or di-ethanolamine) to the balsam which approached nearest to neutrality (*p*H 7·4), we were able to alter its *p*H in the direction of acidity or alkalinity and observe the same effects upon the retention or fading of the stain. Roussy and Lhermitte (quoted in Bolles Lee's *Vade-Mecum*, 1937) found that after Van Gieson's counterstain the sections should not enter any alkaline solution, and they clear such sections in carbol xylol and mount them in Canada balsam saturated with salicylic acid. Masson (1923) also adds salicylic acid to the Canada balsam after his iron haematoxylin, acid fuchsin and aniline blue stain. We observed that salicylic acid is only sparingly soluble in both xylol and Canada balsam, and saturation of a commercial Canada balsam (the *p*H of which was 8·7) with salicylic acid failed to make the mixture acid, its *p*H being altered to 7·1. If the mounting media of Roussy and Lhermitte, and those of Masson, succeeded in preventing fading of their preparations, it appears from our observations that the original Canada balsams which they employed were not strongly alkaline (i.e. they were capable of being nearly neutralized or even rendered slightly acid by the salicylic acid), or, that they were acid or neutral in the first instance, in which case the addition of salicylic acid is superfluous.

Our experience with the fading of Van Gieson preparations when mounted

in alkaline mounting media prompted us to try the effects of mounting preparations treated by other staining techniques in acid, nearly neutral (*p*H 7·4), and alkaline media. The stains employed were haemalum and eosin; Ehrlich's haematoxylin and eosin; Delafield's haematoxylin and eosin; Leishman's Romanowsky stain; toluidin blue; carbol thionin; and methyl-blue eosin (Mann). These preparations have now been mounted in the various media over periods ranging from eight months to two years, and the results may be expressed briefly as follows. In the acid media (*p*H 2·8–6·0) the haemalum, Ehrlich haematoxylin, Delafield haematoxylin, methylene blue (Leishman), toluidin blue, carbol thionin, and methyl blue (Mann) have retained their original depth of colour; eosin retains its colour provided the acidity is not greater than *p*H 4·0; it has distinctly faded in media of *p*H 2·8. In the alkaline media (*p*H 8·6–8·7) the haemalum, Delafield haematoxylin, toluidin blue, methyl blue (Mann), carbol thionin and eosin have distinctly faded; Ehrlich haematoxylin has retained its original colour; methylene blue (Leishman) has undergone slight fading. In the medium which approached neutrality (*p*H 7·4) all the above stains have retained their original colour. The use of a mounting medium with a *p*H value of the same order as that of the stain employed would appear to be ideal, but this would involve the glass electrode estimation of the *p*H values of all the stains involved and the alteration of the *p*H of the mounting medium to suit the particular stain, e.g. by the addition of the acids or alkalies cited above. (We observed in our present work that the addition of these substances to the media in quantity sufficient to alter materially the *p*H of some of the commercial media did not materially modify the refractive index of the media.) But the present study shows that the commonly employed stains show no fading, over a period of two years, in a medium which is approximately neutral; they also retain their colour in the acid media, provided, in the case of eosin, that the acidity is not greater than *p*H 4·0. Owing to the great variations in the chemical constitution of the commercial mounting media in common use, it would be decidedly advantageous if a medium with suitable chemical and physical properties, whose chemical constitution was constant, could be prepared. Such substances are considered below.

SYNTHETIC RESINS AND THEIR APPLICATION TO MICROSCOPY

Within recent years various resins have been synthesized which have chemical and physical properties suitable for their commercial use as artificial plastics for moulding in presses for the production of various transparent articles. Imperial Chemical Industries Limited have developed such a resin, which chemically is a polymerization product of methyl methacrylate. It is marketed in two forms, a powder form ("Diakon"), and precast in sheets or rods ("Perspex"). The uncoloured transparent variety of "Diakon" is made in two forms, granules and powder, the granules being finer than the powder. In the present work it has been found that the powder form is more readily

soluble in xylol than the granules. It is specified (commercially) as "Diakon" D. 100, clear powder.

During the early stages of the present work, E. C. Cole (1938) published an account of his employment of methyl methacrylate polymeride as a mounting medium for histological purposes. He used the sheet or rod form dissolved in chloroform and dioxan. This form is nearly insoluble in xylol. Owing to the poisonous nature, cost and other disadvantages of dioxan (e.g. it must be kept in the dark; it absorbs moisture), we preferred to experiment with "Diakon" powder, which is readily soluble in xylol or benzol.

To prepare "Diakon" powder as a mounting medium, it is dissolved in xylol (4 oz. "Diakon" in 500 c.c. xylol), the process being hastened by gentle heat (incubator at 60° C., in stoppered bottle, with occasional stirring). The medium thus prepared has a refractive index for Na D line at 18° C. of 1·4984 in its liquid form, and $1{\cdot}503 \pm 0{\cdot}005$ when allowed to solidify. When neutral xylol (*p*H 7·0) is employed as solvent, the mixture has a *p*H 4·0. Preparations stained by the methods cited in the former part of this paper have been mounted in this medium. They have retained their original depth of stain over a period of 18 months (to date). As the medium has a constant chemical composition and *p*H, is inexpensive (compared with the commercial mounting media commonly used), has suitable optical properties, is colourless and does not become coloured with age, it was thought in the early stages of the present investigation that this medium would be more suitable for histological purposes than Canada balsam and other commercial media. But, as time progressed a serious obstacle to the use of this medium was observed. When the medium was first placed on the slide and the cover glass added, the medium exhibited marked adhesive properties, the cover being securely fixed to the slide within ten minutes. After a few weeks, however, it was noted in some of the preparations that the solvent (xylol or benzol) gradually began to separate and form small lakes between masses of semi-solid "Diakon". This process of separation of solvent gradually spread and finally the lakes of solvent evaporated leaving spaces which became occupied by air. Further, the adhesive property of the remaining substance was considerably reduced, so that it became possible to dislodge the cover glass from the slide by even gentle side pressure with the finger. In other cases, for a reason unknown to us, this process of separation of solvent did not occur, but even in these cases the adhesive properties of the medium disappeared before the expiry of six months. In other cases, the process of separation of solvent came on rapidly (within a few days) and at first we thought this was due to some fault in our mounting technique, but further observations convinced us that this is an intrinsic fault of the medium and renders it unsuitable as a histological mountant. In all cases, whether separation of solvent takes place or not, the original depth of staining was retained, and this we correlate with the acidity of the medium (as expressed in the first part of this paper).

We have also experimented with a synthetic resin called "Distrene"

(manufactured by the Distillers Company Limited), which is a polystryene with properties akin to those of "Diakon". It is soluble in xylol, colourless, glass clear, and has a *p*H of 6·5. Its refractive index is 1·668. During the progress of our experiments with this substance, Kirkpatrick & Lendrum (1939) observed that whereas mounts prepared by the simple solution of "Distrene" in xylol showed after an interval varying from weeks to months a very marked retraction under the coverslip (similar to the effects we noted in our experiments with "Diakon"), the addition of tricresylphosphate as a plasticizer to the Distrene-xylol mixture obviated this retraction. They further noted this latter mountant preserved the colour of bacteriological and haematological preparations better than Canada balsam. In our experiments we have noted the absence of fading, over a period of 18 months, of histological preparations prepared by the methods cited above when mounted in "Distrene" dissolved in xylol and this again we correlate with the *p*H of "Distrene". But we also noted the separation of solvent, retraction, and air replacement of the evaporated solvent, as described in our observations on "Diakon". Further, as with "Diakon", the mount gradually lost its adhesive properties and the coverslip became very easily dislodged. For these reasons, "Distrene" in simple solution in xylol (or benzol) must be considered unsuitable as a mounting medium. We have made no observations on the addition of a plasticizer to this medium.

Recently, Groat (1939) has published the results of his experiments with two new mounting media, Nevillite V and Nevillite I. These synthetic resins are cycloparaffin polymers (manufactured by the Nevillite Company, Pittsburgh, U.S.A.). While the two are alike in general characters, Nevillite I yellows slightly with age and has a refractive index of 1·567 (compared with that of Nevillite V which is 1·544). Nevillite V is therefore preferable as a mountant. It is now known by its newer name, Clarite. It is inert, water-white, absolutely neutral (we determined its *p*H to be 6·8–7·0 when dissolved in neutral xylol), and has good adhesion to glass. Groat recommends a 60 % solution in 40 % toluene by weight as an excellent medium. While it is soluble in toluol, xylol and benzol, Groat favours toluol as solvent owing to its more suitable volatility. The technicians of General Biological Supply House, Inc., Chicago (*Turtox News*, 1939, **17**, no. 11, p. 143), on the other hand, find xylol more satisfactory than toluol, and note that the medium has a greater tendency than balsam to suck air under the edge of the cover glass as the preparation dries; they add that this drawback is not serious for ordinary mounts and can be overcome with a little experience. We ourselves have found xylol satisfactory as a solvent for Clarite (the substance dissolves more readily in xylol than in toluol) and, provided that ample quantities of the medium are employed, we have not experienced, over 8 months, any separation of solvent comparable with that which occurred with "Diakon" and "Distrene" even in those cases in which we placed the mounts in the incubator at 56° C. to accelerate drying. We use 60 % Clarite in 40 % xylol by weight. This is thinner than a similar strength of balsam. Preparations stained by the methods named in

the former part of this report have been mounted in this medium and have retained their original depth of stain over a period (to date) of eight months. Further, there has been no loss of adhesive properties as in the case of Diakon and Distrene. It appears to us therefore that Clarite is the most satisfactory of the synthetic resins that have been employed as histological mounting media, as far as present observations go. With regard to price, Clarite medium, prepared as above, is slightly more expensive than the ordinary commercial Canada balsam, but is very considerably cheaper than special, so-called "neutral" mounting media.

To sum up, Clarite dissolved in xylol, appears to be a mounting medium which has decided advantages over Canada balsam and other commercial media in that it is quickly and simply prepared from the original crystals, it is colourless and does not become coloured with age, it is inexpensive, it is neutral, inert and its chemical composition is constant. Moreover, its optical properties are suitable for microscopy. With regard to the fading of stained preparations it has been shown in the earlier part of this paper that whereas alkalinity of the mounting medium is deleterious to many stains, neutrality or acidity of the medium, provided that the acidity is not greater than *p*H 4·0, is not harmful. Inert, neutral Clarite is also not harmful to any of the stains tested.

SUMMARY OF CONCLUSIONS

1. Attention is called to the fact that the commercial mounting media commonly employed vary markedly in their chemical constitution, a preparation listed as "neutral" being either acid or alkaline.

2. An acid mounting medium, provided its acidity is not greater than *p*H 4·0, does not cause fading of the commonly employed stains.

3. Experiments carried out with various synthetic resins indicate that Clarite, which is neutral, inert, chemically constant, and suitable optically, has decided advantages over the commercial mounting media commonly used.

I wish to express my indebtedness to Prof. R. P. Linstead, F.R.S. (now of Harvard University), and Dr T. B. Smith, of the Chemistry Department of the University of Sheffield, for their estimations of the *p*H and refractive indices of our materials, and to my laboratory technician, Mr H. Bramham, for the preparation of the histological material which this work entailed.

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THE NERVOUS AND VASCULAR RELATIONS OF THE PINEAL GLAND

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From time to time anatomists have drawn attention to obvious analogies which exist between the epiphysis and the neural part of the hypophysis. Both are developed as diverticula of the diencephalon, both are characterized by cellular elements which are believed to represent a modified type of neuroglia and whose processes have special relations with the walls of blood-vessels, both are apparently related by nerve fibres to adjacent cell collections in the hypothalamus or epithalamus, and there is some evidence to show that both have a secretory function of some kind.

In recent years the nervous and vascular relations of the hypophysis have been studied in considerable detail by the examination of normal material and also by experimental methods. A special vascular relationship between the hypophysis and certain groups of cells in the hypothalamus has been described by Popa & Fielding (1930) and also by Basir (1932), though the conclusions of these authors have been criticized and modified by the more recent researches of Wislocki & King (1936). The existence of efferent fibre connexions from certain cell groups in the hypothalamus (particularly the nucleus supraopticus) has been affirmed by the study of normal silver preparations, and confirmed by experimental anatomical methods and by the study of clinico-pathological material. It therefore becomes a matter of some interest to determine whether the pineal gland is related in a similar way to the epithalamus.

The present investigation was initiated with the study of the pineal gland of the rhesus monkey, of which abundant material has been available in this laboratory. Certain observations on this material led to a further study of the human pineal gland and adjacent structures, and the specimens used for this purpose have been obtained at necropsies.

MATERIAL AND METHODS

For the study of the nervous relations of the pineal gland the following macaque material has been used:

(*a*) Stained with Bodian's protargol technique.

(1) Sagittal serial sections of the pineal gland and adjacent part of the brain.

(2) Horizontal serial sections of the pineal gland and adjacent part of the brain.

(3) Sagittal serial sections through a block containing the pineal gland and the whole length of the straight sinus.

(4) Horizontal serial sections through a similar block.

(*b*) Stained with pyridine silver.

(5) Sagittal serial sections through the pineal gland and adjacent part of the brain.

(6) Sagittal serial sections through a block containing the pineal gland and the whole length of the straight sinus.

(*c*) Stained by Stöhr's modification of Schultz's silver impregnation method.

(7) A spread preparation of the tissue connecting the apex of the pineal gland to the straight sinus.

(*d*) Stained with intravitam methylene blue (0·05 %).

(8) and (9) Spread preparations of the pineal gland, straight sinus and adjacent membranes, cleared in oil of wintergreen.

In addition (10), sagittal serial sections of human material (stained by Bodian's method) were prepared from a block containing the pineal gland, the anterior end of the straight sinus, and the intervening tissue.

For the study of the vascular relations of the pineal gland in the macaque monkey the following material was prepared:

(*e*) (11) and (12) Thick frozen sections of the pineal gland and adjacent part of the brain, in which the blood-vessels were demonstrated by the use of the nitroprusside benzidene method as elaborated by Pickworth (1934). Two such preparations were made.

(*f*) Carmine-gelatin injection.

(13) In one animal in which the whole body had been injected, the pineal gland and adjacent tissues were removed, dehydrated, and cleared in oil of wintergreen.

(*g*) Stained with haemotoxylin and eosin.

(14) and (15) Serial sagittal sections through blocks containing the pineal gland and the whole length of the straight sinus.

(16) Thick celloidin sections through a block containing the pineal gland, anterior end of the straight sinus and adjacent tissues.

Lastly, from human material, serial celloidin sections (cut alternately at 30 and 150 μ) were prepared from a block containing the pineal gland, anterior end of the straight sinus, and adjacent tissues. These were stained with haemotoxylin and eosin, except for a few selected sections which were stained with Van Gieson's stain and orcein.

OBSERVATIONS

Many neurohistologists have recognized the presence of nerve fibres, myelinated and unmyelinated, in the pineal gland of mammals. It is now generally accepted (Hortega, 1932) that these fibres are derived from three sources: (*a*) sympathetic plexuses accompanying the arteries in the adjacent part of the tela chorioidea, (*b*) habenular commissure, and (*c*) posterior commissure. This statement is in accord with observations published by Darkschewitz (1886), Favaro (1904), Marburg (1909), Pastori (1928), Cutore

(1910), Walter (1914), Achúcarro & Sacristán (1912), Josephy (1920), Pines (1927), Antonow (1925), etc. Some anatomists have suggested definite nuclear and fascicular origins for the fibres which enter the pineal gland from the habenular and posterior commissures—such as the habenular ganglion, the tectum of the midbrain, the fasciculus retroflexus, the taenia thalami, the intercalary tract, and even (Darkschewitz) the optic tract. Perhaps the most ambitious account of the fibre connexions of the gland is that recently provided by Roussy & Mosinger (1938), in which they attempt to bring the structure into their scheme of the "neuro-endocrine" system of the diencephalon, and to link it up functionally and anatomically with the neural lobe of the pituitary gland. According to this account, the fibres in the pineal gland are derived from (among other sources) the habenular ganglion, the anterodorsal nucleus of the thalamus, the nucleus tangentialis accessorius, the preoptic area of grey matter, the periventricular grey matter of the thalamus, and the superior and inferior colliculi. Such conclusions appear to be based entirely on the casual inspection of sections of normal material; for this reason they must be regarded as entirely uncertain, simply because there is no possibility in such sections of tracing individual fibres in continuity over these distances.

In regard to the distribution of nerve fibres within the substance of the pineal gland, there has been some difference of opinion. Cutore, for example, described the commissural fibres as spreading out into the pineal parenchyma in a fan-wise manner to contribute to the formation of the plexus in the main part of the gland and almost reaching its apex, while Walter supposes that the epiphysis contains but a small component of fibres from the habenular and posterior commissures. On the other hand, Cajal takes the view that the nerve plexus in the main body of the gland is entirely derived from sympathetic fibres accompanying the chorioidal vessels. Those fibres which previous authors had attributed to a commissural origin are really derived, according to Cajal, from plexuses accompanying inferior pineal arteries which enter the gland near its base and are directed distally. Incidentally, Cajal recorded a very rich plexus of nerve fibres in the pineal gland of the mouse, and he described their terminal ramifications as forming an extremely fine network surrounding clusters of pineal cells and recalling in appearance the nerve plexus found in the pancreas and salivary glands. On the other hand, Antonow (1925), studying the pineal gland of the dog, cat, rabbit and deer, was unable to find the dense network of fibres described by Cajal, and supposed that the latter had misinterpreted the appearance of reticular connective tissue fibres. Such a criticism, in view of Cajal's great reputation, appears likely to be unfounded. Pines (1927) has more recently described and figured nerve endings in the pineal gland of the dog, showing minute end-bulbs apparently related to the parenchymatous cells. Also, since he found that the terminal network has no direct relationship with blood-vessels, he concludes that it represents part of a nervous apparatus which stands in immediate relation to the specific functions of the epiphysis—in other words, it is composed of secretory fibres.

When silver-impregnated sections of the macaque pineal gland (which had been prepared for the present study) were first examined, numerous cells were found in the centre of the gland of whose nervous nature there could be no doubt (Pl. II, fig. 3). This observation occasioned some surprise, since it is generally supposed that true nerve cells are not to be found within the substance of the epiphysis except as occasional heterotopic elements (Hortega, 1932). The cells are relatively large, usually multipolar (but sometimes bipolar), and give rise to numerous branching processes. They are mainly collected in the centre of the distal half of the pineal body and are here embedded in a mass of neuropil which forms a central core to the gland (Pl. I, fig. 2). Occasional cells of a similar type are also to be found in the proximal half of the gland.

A reference to the relevant literature showed that these nerve cells had been previously recognized and described. Kolmer (1929) recorded the presence of relatively large nerve cells in three species of monkey, *Macaca mulatta*, *M. sinica*, and *Papio cynocephalus*. He found that they were embedded in a layer of neuroglia and connective tissue in the distal part of the pineal gland, and from this region he was able to trace myelinated fibres, accompanying small blood-vessels, towards the tip of the gland where they emerge on the surface. Here the fibres form a well-defined bundle, lying in relation to the vena magna cerebri, which Kolmer (in collaboration with Loewy, 1922) had previously described in certain lower mammals (i.e. guinea-pig, dog and goat). This nerve bundle has been named the *nervus conarii*,[1] and it was Kolmer and Loewy who first suggested that it contains efferent fibres whose cells of origin lie in the pineal gland itself. Later the nerve was studied by Pastori (1930), who recorded it in the dog, cat, sheep, monkey and man. He also described as a constant feature in all these species a circumscribed group of small nerve cells situated in the pial tissue close to the tip of the pineal gland, and which he termed the *ganglion conarii*. In a series of nine human brains he claims to have identified this ganglion, and states that it contains about twenty nerve cells, closely packed, and 15–20 μ in size. Unfortunately Pastori does not in his paper offer a photomicrograph for inspection to show the appearance of these cells. It may be noted, however, that a ganglion in this position was described many years previously by Marburg (1909) in the brain of a new-born infant, and this author compared it to the parietal ganglion of reptiles.

More recently, a short paper has appeared by Levin (1938) calling attention once more to the presence of numerous ganglion cells within the pineal parenchyma of monkeys (*Macaca mulatta*, *M. radiata*, *Ateles ater*, and *Alouatta seniculus*). In the bonnet monkey a count showed the number of ganglion cells to be approximately 2000.

Apart from monkeys, however, no true nerve cells have been certainly identified in the substance of the pineal gland except possibly as occasional heterotopic elements, and according to Pastori they are also absent in the

[1] In the literature the word "conari" is used, but this is clearly incorrect.

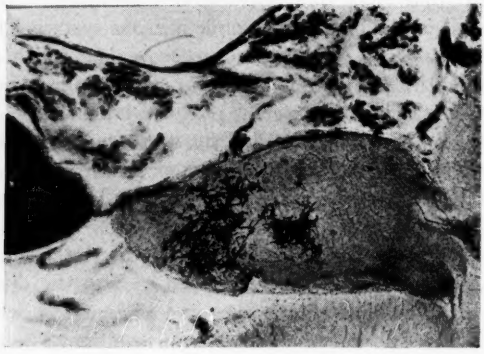
chimpanzee and in the marmoset (*Hapale jacchus*). The possibility at once suggests itself that the intrapineal ganglion cells of monkeys represent the ganglion conarii of Pastori which has become secondarily incorporated within the substance of the gland instead of occupying the usual position in the pial tissue at its apex. However, the cells within the macaque pineal gland appear to be much more numerous and also considerably larger than those described in the ganglion conarii of other mammals by Pastori.

As already noted, the silver-impregnated macaque material which was prepared for the present study has fully confirmed the presence of many ganglion cells in the pineal gland of these animals. The cells show no regular orientation, most of them give off a number of stout branching processes which contribute to and are soon lost in the dense plexus of nerve fibres in which they lie, and in general appearance they bear a close resemblance to the nerve cells found in peripheral ganglia of the autonomic nervous system. The central core of neuropil in the macaque epiphysis is very vascular, and herein a marked contrast is shown with the more proximal part of the gland where the vascularity is by no means rich. The vascular plexus related to the central core of neuropil may be seen by reference to Pl. I, fig. 2, where the capillary vessels are seen as clear areas in the mass of interlacing nerve fibres. It is also quite well shown in ordinary haemotoxylin and eosin sections. In the specimen of the macaque pineal in which the blood-vessels had been injected with carmine gelatin, it is perhaps most clearly evident. Here the closely meshed capillary plexus can be seen to occupy a circumscribed area in the distal half of the gland, corresponding in position to the core of neuropil which had been demonstrated in other specimens by silver impregnation. The plexus receives its main contribution from a leash of fine vessels entering the pineal gland at its apex (to which future reference will be made), but it also receives accessions from vessels lying on the surface of the distal half of the gland and penetrating the latter from the lateral aspect. The high vascularity of the neuropil core is presumably related to the level of its metabolic activity, and, in view of recent studies by Dunning & Wolff (1937), the existence within this network of synaptic arborizations seems probable. It is interesting to note, also, that in the same region of the pineal gland in the monkey thick sections show a local accumulation of large and elaborately branching melanocytes, filled with dark brown pigment (Text-fig. 1). The presence of melanin pigment in the pineal gland of mammals is well recognized, and Roussy & Mosinger (1938) have recently made use of this observation to extend their conception of "pigmentary neurocrinie".

We may now consider the origin of the nerve fibres which can be seen in silver-impregnated sections to penetrate the pineal gland and apparently to take part in the formation of the network of nerve fibres within its substance. These fibres may conveniently be divided into three groups: (*a*) those which enter from the lateral aspect of the gland in company with blood-vessels, (*b*) those which enter by way of the peduncle of the gland, and (*c*) the con-

spicuous fasciculus which can be seen emerging from (or penetrating) the apex of the gland.

(*a*) The small arterioles which enter the surface of the pineal gland in the monkey are particularly well supplied with nerve fibres which run in close contact with them; indeed, their innervation is considerably more rich than that of cerebral vessels entering the brain substance. They are essentially components of the chorioidal system of vessels, being terminal branches of the posterior chorioidal arteries, and it has previously been emphasized (S. L. Clark, 1934) that vessels supplying chorioidal plexuses have an abundant nerve supply. Within the gland, vascular nerve fibres frequently run as discrete fasciculi in direct relation to the blood-vessels, and many of them become lost



Text-fig. 1. Thick sagittal section of the pineal gland of a rhesus macaque, showing the distribution of melanocytes in the parenchyma. Above is the dorsal sac containing tufts of chorioidal tissue. To the left is seen the vena magna cerebri receiving a small venous tributary from the pineal gland. × 17.

in the central nerve plexus so that their ultimate destination cannot be determined. The sections provide no evidence that any of the fibres have a direct terminal relation to the parenchymatous cells of the epiphysis.

(*b*) Sections of the pineal gland stained with silver nitrate seem to confirm the observations of many workers that bundles of fibres from both the habenular and posterior commissures contribute to the innervation of the parenchymatous tissue of the gland. These bundles are also to be seen in Weigert sections, particularly in the pineal gland of larger mammals, and they give the appearance of breaking up into a somewhat diffuse radiating network terminating in the proximal part of the gland. A closer study of serial sections, however, indicates that this appearance may be quite illusory.

The fibres entering the pineal peduncle from the habenular commissure arise in part from cells of the habenular ganglion. Of this there can be little doubt, for processes of the cells can be seen to pass directly into the fasciculi

which contribute to the formation of the commissure. Other fibres are probably derived from the taenia thalami and may have a more distant origin.

There can be no question of the fact that fasciculi of these commissural fibres enter the proximal part of the gland. If, however, individual fasciculi are followed section by section, in some instances they can be observed to arch medially, and then to run down and leave the gland again, rejoining the habenular commissure on the opposite side. In their course the individual fibres of these fasciculi may become frayed apart by the pineal cells among which they course, subsequently reforming again into a discrete fasciculus. It is thus apparent that these fibres have no terminal relation to the pineal parenchyma—they are merely aberrant commissural fibres. The question now arises whether this interpretation is true of all the fibres of the habenular commissure which can be seen in individual sections to enter the gland. This is clearly difficult or impossible to prove. But it appears certain that, in the embryonic outgrowth of the pineal diverticulum, certain fibres of the habenular commissure have become caught up and displaced, so that they come to pursue an aberrant and broken-up course among the parenchymatous cells of the gland, and it is not improbable that all the nerve fibres which are seen to enter the proximal part of the epiphysis are of this nature. Some of the fasciculi of the commissural fibres, however, meet and become merged with vascular nerve fibres which enter the gland at a higher level and are directed downwards. In this case, an *appearance* of continuity may have led many observers to affirm that fibres of the habenular commissure extend throughout the greater part of the pineal gland, and actually terminate therein.[1] Incidentally, it is possible that the "nervus parietalis", described by Marburg (1920) in the ventral wall of the suprapineal recess of an antelope, is simply a part of the habenular commissure which has been drawn out into a much elongated loop by the outgrowth of this sac during its embryological development.

The true relation of the posterior commissure to the pineal gland appears to be the same as that of the habenular commissure. Its fibres are drawn up into the posterior wall of the pineal recess, and reach into the base of the gland. Among them may also be found a few small fusiform nerve cells—evidently heterotopically displaced cells from the nucleus of the posterior commissure. Although vascular nerve fibres descending in the gland become intermingled with posterior commissural fibres, a careful study of the serial sections makes it appear probable that the commissural fibres loop up only to return to the opposite side of the posterior commissure, and play no part in the innervation either of the pineal parenchyma or of the vessels of the pineal gland. There is one certain exception to this general conclusion—a few fibres from the

[1] It is relevant to note that in other parts of the brain the displacement to an aberrant position of some of the fibre components of a single tract has on occasion certainly led to a similar misinterpretation. This is the case, for example, with the so-called "anterior accessory optic tract", and also with fibres of the optic chiasma which may turn dorsally into the periventricular region of the hypothalamus.

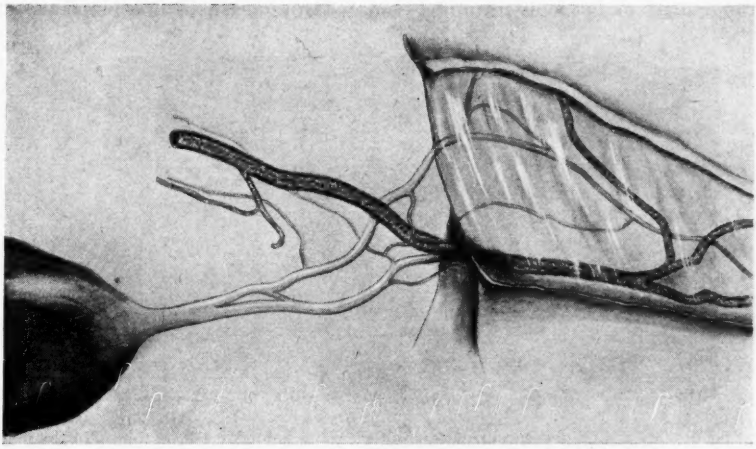
posterior commissure leave the aberrant fasciculi and ramify immediately beneath the ependymal epithelium lining the posterior wall of the pineal recess. These really appear to be terminal fibres, and they show a characteristic regular beading along their course just before they terminate, as well as a minute end-swelling. Similar bulbs have been described by Pines (1927) on nerve fibres in the substance of the pineal gland in the dog, and he regards them as evidence of a secreto-motor function. In our own material, such bulbs can also be seen here and there on fibres in the pial tissue investing the gland, and in the pial septa within the gland. But where they have at first sight appeared to be present on fibres lying in relation to parenchymatous cells, this appearance has been found on more careful examination to be illusory only, and due either to a sudden bend or kink in a nerve fibre (simulating a local or terminal thickening), or to the superimposition of a fibre on the darkly stained nucleolus of one of the cells.

The general conclusion to be drawn from this study, i.e. that the fibres of the habenular and posterior commissures which enter the pineal gland are merely aberrant commissural fibres, and have no functional relation to the pineal parenchyma, is consonant with certain other observations. Herring (1927) has drawn attention to the fact that in the rat the pineal gland is separated by some distance from the region of the habenular and posterior commissures, and in this case fibres from the latter apparently do not reach the substance of the gland at all. The same is the case for detached lobules of the gland which may occasionally be seen in other animals. In the serial sections of the pineal region of the human brain prepared for this study by silver impregnation, a small isolated lobule of pineal tissue was found embedded in the dorsal wall of the vena magna cerebri some distance from the main gland, and a study of the sections showed no nerve fibres entering it from the latter. Lastly, even those observers who have convinced themselves that the commissural fibres do in fact terminate in the gland in most cases agree that these fibres only reach the proximal part. It would be unusual, however, for the proximal part of the pineal parenchyma to receive a secretory nerve supply different from the distal part, since its intrinsic structure is everywhere the same.

(*c*) Silver-impregnated serial sections of the pineal gland of the macaque monkey show that from the central core of neuropil a strong fasciculus of nerve fibres proceeds distally to the apex of the gland and there leaves the latter to course back along the ventral wall of the vena magna cerebri (Pl. I, fig. 1). In this situation it is enclosed in a connective tissue sheath (derived from the pia mater), and in appearance and compactness, as well as in the presence of neurilemma cells, it resembles a peripheral nerve. Reference to the literature shows that this nerve bundle is identical with that described by Kolmer and Loewy under the name *nervus conarii*. The origin and direction of this nerve are quite uncertain. The sections suggest that its fibres may be efferent with respect to the gland, and that they may be derived from the

axonal processes of the ganglion cells already described. Such an inference, however, requires experimental investigation in order to allow of certainty. The fibres are, at least in part, myelinated, and as Levin (1938) has pointed out, this suggests that they are not derived from the cervical sympathetic ganglia, since post-ganglionic fibres are commonly unmyelinated.

The further course of the nervus conarii was studied by three methods: (1) by following it under the dissecting microscope in the tissues of this region which had been removed from monkeys stained intravitally by the injection into the circulation of 0·05 % methylene blue, (2) by the examination of silver-impregnated serial sections of a block containing the pineal gland, the whole



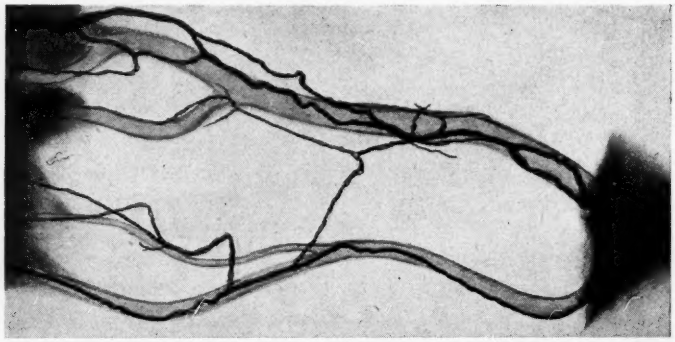
Text-fig. 2. Drawing of a dissection showing the nervus conarii in a rhesus macaque. The tissues were stained by the intravitam injection of methylene blue. From the tip of the pineal gland fasciculi of nerve fibres extend into the wall of the straight sinus (which has been opened up from the left side) and here lie in close relation to branches of a chorioidal artery. ×30 (approximately).

length of the straight sinus, the vena magna cerebri, and immediately adjacent tissues, and (3) by tracing the fibres in spread preparations stained by Stöhr's modification of Schultz's silver impregnation method.

Two methylene-blue preparations were made, and Text-fig. 2 represents a drawing made of one of these with the aid of a binocular dissecting microscope. The nerve was readily found by this method, issuing from the top of the pineal gland as a well-defined strand. This was teased apart from the tissues in which it lay, and it could be followed running a slightly plexiform course to the commencement of the straight sinus. Here it gave rise to branches which entwined a small arteriole in this region (a branch derived from one of the posterior chorioidal vessels), and some recurrent twigs which followed the

arteriole and one of its branches. The nerve then entered the dural wall of the straight sinus and could be followed for some distance in a subendothelial position—always in fairly close relation to the arteriole (which was also found to ramify in the wall of the sinus—*vide infra*). The subsequent course of the nerve could not be followed further in these preparations owing to the density of the dura mater.

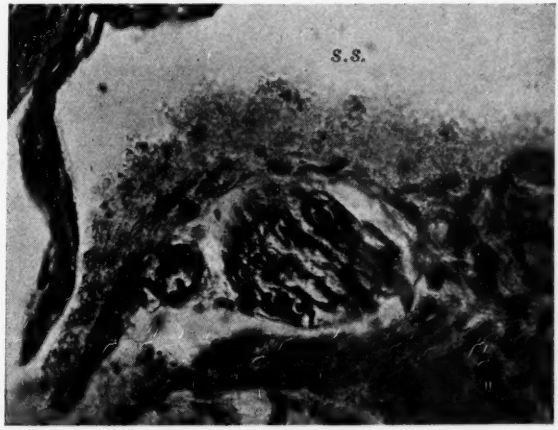
In the silver-impregnated serial sections, the strand of nerve-fibres recognized in the dissections can be seen as a circumscribed bundle emerging from the tip of the pineal gland and passing backwards in close relation to the ventral wall of the vena magna cerebri (Pl. I, fig. 1). In its course towards the commencement of the straight sinus it is seen to break up into a plexiform arrangement of fasciculi intimately related to a leash of fine blood-vessels



Text-fig. 3. Drawing of a spread preparation of the tissues extending from the tip of the pineal gland to the anterior end of the straight sinus in a rhesus macaque, stained by Stöhr's modification of Schultz's silver-impregnation method. In the drawing the apex of the pineal gland is to the left and the dural wall of the straight sinus to the right. The fasciculi of nerve fibres are seen to be closely associated with small blood-vessels which descend to the tip of the gland. × 60 (approximately).

which run down to enter the tip of the pineal gland. It thus appears that the nerve fibres may be primarily vasomotor in type, and this possibility is certainly suggested by their appearance in a spread preparation stained by Schultz's silver-impregnation method. In this preparation, which is illustrated in Text-fig. 3, the strand of tissue connecting the apex of the pineal gland with the tentorium cerebelli has been teased apart, and the blood-vessels and nerve fasciculi have been separated instead of forming a compact bundle. To the left of the figure is the apex of the pineal gland, and here the nerve fibres, splayed apart, can be seen emerging alongside the vessels. To the right is the dense dural tissue at the commencement of the straight sinus, into which the nerve fibres disappear. The opacity of the dura mater prevents them from being followed further.

Reverting once more to the silver-impregnated serial sections of the macaque pineal and adjacent tissue, it can be seen that bundles of fibres derived from the nervus conarii actually run distally for a considerable distance in the wall of the straight venous sinus, occupying here a subendothelial position (Text-fig. 4). They are frequently accompanied by small arterioles, and it is thus possible that they are related functionally more directly to these vessels than to the straight sinus itself. While the preparations are not adequate for the demonstration of the finer nerve endings, in some sections there is to be seen in the connective tissue between the pineal gland and the straight sinus a globular network of nerve fibres closely similar to the tangled skeins of varicose fibres which S. L. Clark (1934) describes as nerve endings in the chorioid plexus, and which he likens to Meissner's corpuscles.



Text-fig. 4. Transverse section through the anterior end of the straight sinus of a rhesus macaque, stained by Bodian's protargol method. A fasciculus of nerve fibres occupying a subendothelial position is shown in the wall of the sinus. ×200. *s.s.* = lumen of straight sinus.

Whatever the true nature may be of the nervus conarii in the macaque monkey, it seems certain that it is closely related to the blood-vessels of the pineal gland and adjacent tissues, and it becomes necessary to enquire into the origin and course of the latter, with particular reference to the arterial twigs which run up from the region of the pineal gland to enter the dura mater in the wall of the straight sinus.

In the course of dissections of the pineal gland of the monkey, including both injected and uninjected specimens, it was found to be vascularized entirely from chorioidal vessels derived eventually from the posterior cerebral arteries. As seen in Text-fig. 5, on each side a chorioidal artery runs distally in close relation to the lateral surface of the gland. In its course, it gives off two or three small branches which may either penetrate the gland directly (particu-

larly near the base of the gland), or may ramify in a rather tortuous course in the pial investment of the gland before its terminal branches enter the gland substance. Moreover, cross-connexions may exist, running transversely over the front and back of the gland and joining together the main chorioidal vessels of either side. The further course of the main chorioidal vessels is somewhat unusual. Just above the tip of the pineal gland they may (but do not always) unite to form a single small arteriole which is continued alongside the nervus conarii (i.e. on the lower wall of the vena magna cerebri) and enters the dura

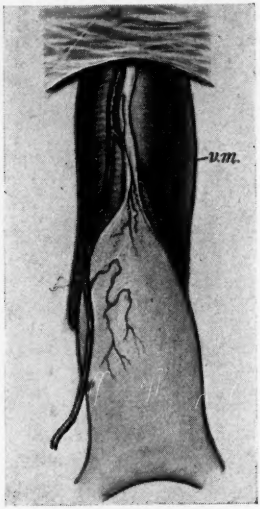


Fig. 5

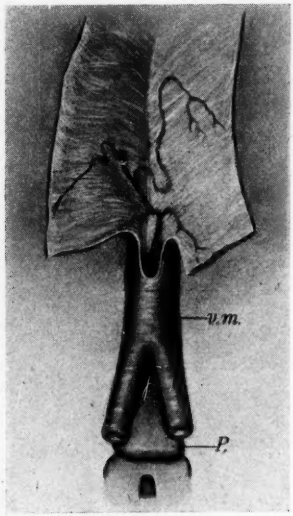


Fig. 6

Text-fig. 5. Drawing of a dissection showing the nervus conarii of a rhesus macaque, extending from the tip of the pineal gland into the dural floor of the straight sinus—viewed from the ventral aspect. A small artery formed by the coalescence of two chorioidal vessels follows the same course. ×10. *v.m.* = vena magna cerebri.

Text-fig. 6. The same specimen as that in the previous figure, viewed from the dorsal aspect. The straight sinus has been opened to show the artery ramifying in its floor and lateral walls. ×5. *P.* = pineal gland; *v.m.* = vena magna cerebri.

mater forming the floor of the straight sinus (Text-fig. 6). On its way, it gives off a leash of fine descending arterioles which penetrate the substance of the pineal gland at its apex, and contribute largely to the rich capillary plexus pervading the neuropil core in the macaque epiphysis. In its further course, the main vessel can be followed into the floor of the straight sinus where it breaks up into rather tortuous terminal branches ramifying in a subendothelial position. It is in relation to these branches that the fibres of the nervus conarii so closely run. The intradural course of the artery has been followed in two series of serial sections stained with haemotoxylin and eosin, and in one

specimen a small branch (with an accompanying fasciculus of nerve fibres) was found to traverse the lumen of the straight sinus in a trabecular strand of dural tissue.

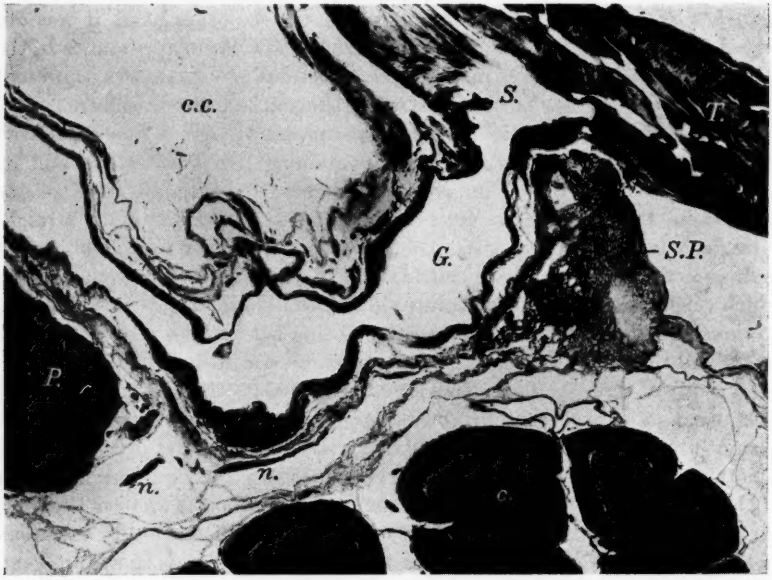
In order to enquire whether a similar vascular arrangement might exist in relation to the human pineal gland, two brains were carefully removed at autopsies together with the dura mater wall of the straight sinus. The posterior cerebral arteries were injected with carmine-gelatin through the basilar artery, and the main chorioidal branches were followed by dissection. In neither case, however, were macroscopic arterial twigs traced from any of these branches into the wall of the straight sinus. On the other hand, it was observed that a granular tuft of arachnoidal tissue, similar in appearance to the Pacchionian bodies found in many of the intradural venous sinuses, is present in close relation to the floor of the straight sinus at its commencement.

A complete series of serial sections was prepared from a block removed from a human brain (female, aet. 47) containing the pineal gland and its attachments to the mid-brain and epithalamus, the adjacent part of the cerebellum, the vena magna cerebri, the anterior extremity of the straight sinus, and neighbouring tissues. The block was embedded in celloidin after preliminary treatment with 5 % nitric acid to decalcify any calcareous deposits which might be present in the pineal gland and chorioid plexuses. Sagittal sections were prepared, alternate sections being cut at thicknesses of 30 and 150 μ, and these were stained with haemotoxylin and eosin.

A study of these sections shows a number of points of exceptional interest. The pineal gland itself contains several large calcareous masses (*acervuli*) with a concentric laminated structure, and also a large cystic cavity of a type which is not uncommonly found here. The nervus conarii is clearly evident and in one of the thick sections its uninterrupted course can be followed up from the tip of the pineal gland along the inferior wall of the vena magna cerebri for a distance of over 6 mm. Its appearance in the adjacent thin section is shown in Text-fig. 7 (*n.n.*). The diameter of the nerve measures 0·1 mm. in section. Within the pineal gland the nerve fibres become dispersed along interlobular septa, and their precise destination cannot be followed. Above the gland the nerve pursues an unbranched course which takes it into the dural wall of the straight sinus, and on its way it passes through a somewhat remarkable formation of the pia-arachnoid. This formation resembles a single large and globular arachnoid granulation, projecting up from the surface of the superior cerebellar vermis. It reaches to, and slightly invaginates, the floor of the junctional region between the vena magna and the straight sinus (Text-fig. 7). At its summit the granulation is adherent to the dural floor of the sinus, but elsewhere it is separated from the dura mater by the subdural space. It measures 4 mm. in height and 2·5 mm. in width at its attached base.

The structure of the granulation is peculiar. Within it is a stroma of unusually dense pial tissue, which forms an interlacing reticulum of well-defined fibrous trabeculae. In the meshes of the latter is a rich plexus of sinusoidal

blood-vessels, with several large interconnecting blood sinuses. These cavities are lined by a single-celled layer of endothelium which directly covers the fibrous trabeculae. The large sinuses are disposed mainly in the anterior half of the granulation—where the latter lies in contact with the wall of the great vein of Galen (Text-fig. 7). From this description it will be apparent that in its general appearance the whole structure shows some resemblance to typical erectile tissues. It does not, however, contain any elastic or plain muscle tissue.[1]

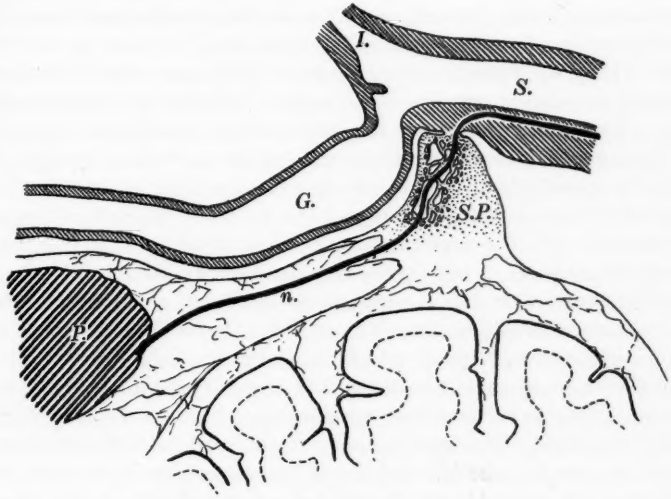


Text-fig. 7. Sagittal section of the pineal gland, vena magna cerebri and adjacent tissues from a human brain. The suprapineal arachnoid body, containing many blood sinuses, is seen projecting up from the surface of the superior cerebellar vermis to abut against the opening of the straight sinus. In adjacent sections the body is seen to be attached directly to the dura mater. ×9. *c.* superior vermis of cerebellum; *c.c.* space occupied by the splenium of the corpus callosum; *G.* great vein of Galen; *n.n.* nervus conarii; *P.* tip of pineal gland; *S.* commencement of straight sinus; *S.P.* suprapineal arachnoid body; *T.* tentorium cerebelli.

The functional significance of this vascular arachnoidal granulation can, at the moment, only be tentatively inferred from its anatomical appearance and disposition. In Text-fig. 7 it will be seen that the blood sinuses within the granulation of this particular specimen are partly empty and collapsed. Yet, even in this condition, the structure appears to elevate the floor of the anterior end of the straight sinus and to narrow the opening of the vein of Galen. If it is

[1] Some of the spaces seen in this arachnoid structure are evidently subarachnoid spaces containing cerebrospinal fluid, and are to be distinguished from the endothelial-lined blood spaces.

recognized that the straight sinus is held rigidly in the taut tentorium cerebelli, it seems more than probable that, in a distended state with the sinusoidal cavities filled, the granulation will force up the dural wall of the straight sinus where this forms the lower lip of its opening until it becomes approximated to the upper lip (which here forms rather a sharp angle with the dorsal wall of the vein of Galen). In other words, the granulation seems to provide a ball-valve mechanism which controls the entry of venous blood from the great vein of Galen into the straight sinus. For descriptive convenience the granulation may be termed the "suprapineal arachnoid body", because of its topographical position above and behind the pineal gland.



Text-fig. 8. Diagram (constructed from serial sections) showing the course of the nervus conarii in the human brain. *G.* great vein of Galen; *I.* inferior sagittal sinus; *n.* nervus conarii; *P.* pineal gland; *S.* straight sinus; *S.P.* suprapineal arachnoid body.

The nervus conarii enters the base of the suprapineal body at a distance of 6·5 mm. from the tip of the pineal gland. It runs a winding course through the body without (so it appears from the sections) giving off any branches, and it actually traverses the cavity of one of the blood sinuses, being ensheathed here with a fine investment of pial tissue. At the summit of the body the nerve runs into the dura mater. Here, after passing through the cavity of a venous lacuna, it turns backwards and courses in a subendothelial position in the floor of the straight sinus (Text-fig. 8). The sections allow it to be followed in this position for 6 mm.; its further course has not yet been followed. The whole length of the nervus conarii, so far as it can be followed in these serial sections, is just 20 mm.

It may be noted that the nervus conarii is accompanied into the dura mater

by a minute arteriole (derived from one of the chorioidal vessels), but this is considerably smaller than the nerve. It is therefore quite accessory to the latter. In the whole course of the nerve no evidence was found for the existence of the "ganglion conarii" of Pastori.

In silver-impregnated serial sections of the pineal gland and adjacent tissues of another human brain (male, aet. 25), the suprapineal arachnoidal body is particularly obtrusive, for the channels of its sinusoidal plexus are filled with blood. Its relation to the straight sinus and the great vein of Galen, however, are not so clear, since the tissues have become somewhat disorientated in the process of fixation and embedding. Also, the granulation has been sectioned more or less transversely to its long axis. It appears as an ovate body, measuring 2·5 by 1·0 mm., and it can be seen to be insinuated among the dense fibrous strands of the dura mater which form the floor of the straight sinus (Pl. II, fig. 4). From the convex surface of the main part of the granulation a small secondary lobule is seen to project. As in the first specimen already described, the pial tissue forming the matrix of the granulation is more dense and less cellular than usual, and it is permeated by a network of irregular blood vessels of a sinusoidal appearance, showing frequent dilatations. In addition, there are to be seen in the granulation a few arterioles of small calibre with a thin muscular wall. The suprapineal body is outlined by a narrow subdural space which, however, is not everywhere distinct. In the dural tissue which immediately surrounds it is a conspicuous bundle of nerve fibres, which is evidently the nervus conarii already described in the previous specimen. In the present case, however, its relation to the pineal gland is not ascertainable since the serial sections do not cover all the field necessary for following its course. This is partly due to the fact that the pineal gland has been considerably distorted by the presence of a large cystic cavity. At one level in the serial sections, however, the nerve can be followed across the subdural space to enter the substance of the suprapineal body. Here it bears a close relation to the sinusoidal plexus, and traverses the cavity of one of the blood sinuses. In sections where the nerve has been cut transversely it is possible to enumerate about 150 nerve fibres in it. Following the nervus conarii section by section through this silver-impregnated material, it was not found to branch, nor did any individual fibres leave the main fasciculus to enter the surrounding tissues either in the suprapineal arachnoid body or in the dura mater in the floor of the straight sinus. In the latter situation (as in the previous specimen) it was seen to traverse the cavity of a venous lacuna.

In the substance of the pineal gland fine nerve plexuses are to be seen on the walls of some of the blood-vessels, but within the too narrow limits covered by the serial sections no continuity could be established between these and the fibres of the nervus conarii within the suprapineal granulation. No dense mass of nerve fibres similar to that observed in the substance of the macaque epiphysis is present in the part of the gland preserved, nor are any nerve cells seen. Also, a careful scrutiny of the sections failed to disclose a "ganglion conarii".

It has already been noted that, except for the capillary network pervading the central core of neuropil in the rhesus macaque, the pineal gland of this species is not conspicuous for its vascularity. An examination of frozen sections of macaque material, stained by the nitroprusside-benzidene method, was made in order to determine whether the gland is connected with groups of nerve cells in the epithalamus by a vascular mechanism similar to that which has been described by some authorities as existing between the pituitary gland and the hypothalamus. No such vascular connexion appears to exist—indeed the peduncle of the pineal gland contains only a few small capillary vessels.

DISCUSSION

The observation that in the pineal gland of monkeys there exists a central core of neuropil containing an abundant number of large ganglion cells again raises the question of the relation of this organ to the nervous system. It must be emphasized that this neural component seems to be a pithecoid peculiarity, and the only structure which might be comparable to it in other mammals is the "ganglion conarii" as described by Pastori. According to him the ganglion is to be found in the pial tissue close to the tip of the pineal gland in a variety of mammals, including Man. These observations have not been confirmed, however (apart from an isolated observation previously made by Marburg in the brain of a new-born human infant), and in the present study such a ganglion was not found in serial sections of two human pineal glands and adjacent tissues, nor in any of the macaque material. In any case, according to Pastori the ganglion is extremely small, for he states that in Man it consists of about twenty cells only. On the other hand, in the monkey the nerve cells within the pineal gland may amount to over two thousand (Levin, 1938), and they are relatively large and provided with numerous processes. It seems improbable, therefore, that they represent the "ganglion conarii" of other mammals which in this instance has become incorporated within the pineal substance instead of being situated near its apex.

The possible significance of Pastori's "ganglion conarii" is quite obscure. He describes the cells as giving off two sets of processes—one set proceeding distally to ramify and terminate in the wall of the vena magna cerebri, and the other entering the pineal gland at its tip in company with some small blood vessels. This disposition of the main fibres suggests a vascular reflex mechanism specifically related to the vessels of the pineal gland on the one side and to the great vein of Galen on the other. It remains possible, also, that if the "ganglion conarii" really exists it may be a functional centre for the control of a vascular mechanism such as that which has been described in the present report in the human brain under the name of the suprapineal arachnoid body. It has yet to be shown, however, that a vascular arachnoid granulation of this type exists in relation to the straight sinus in other mammals. Investigations along these lines are now proceeding. In connexion with this discussion it is relevant to note that a few ganglion cells have been observed elsewhere in the pial tissues

covering the surface of the brain (S. L. Clark, 1931), while the presence of nerve plexuses in relation to pial vessels generally is well established, the fibres being particularly numerous on the chorioidal arteries.

It has been shown in the present communication that, while the fibres of the nervus conarii extend along the wall of the vena magna cerebri, there is no evidence that they terminate there; on the contrary, they actually run an uninterrupted course to enter the dura mater, and then run backwards, occupying a subendothelial position, in the floor of the straight sinus. In the monkey they are closely related to branches of chorioidal arteries which ramify in the wall of the straight sinus. It is possible, therefore, that in this animal they may be vasomotor nerves of the ordinary type.

The origin of the fibres of the nervus conarii still remains in doubt. In the macaque monkey they can be traced along small blood-vessels into the central mass of neuropil in the pineal gland, and possibly they arise from the ganglion cells situated here. The general appearance of the nerve and its branchings as these are seen in dissected specimens certainly suggests that the nerve is efferent with respect to the pineal gland. Also, as already remarked by Levin, the fact that a proportion of the fibres leaving the pineal gland in the monkey are rather heavily myelinated suggests that they are unlikely to be post-ganglionic fibres derived ultimately from cervical sympathetic ganglia. In this connexion it is relevant to note that pial blood-vessels in general may be accompanied by nerve bundles of which some fibres are myelinated, and that nerve fibres have been observed to enter the pia mater directly from cranial and spinal nerve roots (S. L. Clark, 1929, 1931). Further, it has been demonstrated by Chorobski & Penfield (1932) that the perivascular nerve bundles related to cerebral blood-vessels are not appreciably affected by a complete removal of all the sympathetic fibres which accompany the carotid and vertebral arteries into the skull, and also of the greater superficial petrosal nerves. In other words, "it is not possible to denervate the cerebral vessels by removing both sympathetic and parasympathetic innervation".[1]

From the central mass of neuropil in the macaque pineal gland, strands of fibres can also be traced to the more proximal part of the gland in relation to blood-vessels, and here they intermingle with bundles of nerve fibres which enter the lateral aspect of the gland with branches of the chorioidal arteries. At the base of the epiphysis these plexiform fibres come into relation and freely intermingle with fibres from the habenular and posterior commissures which can be seen entering the pineal stalk. It is this intermingling which makes so difficult or impossible the tracing in serial sections of fibres from the commissures. There has never been any doubt that the pineal gland contains nerve fibres derived from perivascular plexuses accompanying chorioidal vessels, and these have commonly been regarded as derivatives of the sympathetic nerves

[1] It is certain that by omitting to take into account this important fact some physiologists have been led in the past to underestimate the efficacy of the vasomotor control of the cerebral arteries.

accompanying the carotid and vertebral arteries. On the other hand, there has been considerable divergence of opinion regarding the contribution of the habenular and posterior commissures to intrapineal plexuses. In the present study it has been established that at least a proportion of these commissural fibres enter the gland on one side of the peduncle, only to leave it by the other side and rejoin the commissures. Moreover, the component fibres of these aberrant fasciculi may become splayed apart and then run a tortuous course among the parenchymatous cells of the pineal gland, so that the fasciculi give an appearance in single sections of breaking up into a spray of terminal fibres. It is not possible, of course, to affirm that all the commissural fibres which enter the pineal parenchyma behave in this way, but these observations allow a fair presumption that this may be the case.

The unusual course of the chorioidal vessels related to the pineal gland of the rhesus macaque, whereby they extend actually into the wall of the straight sinus and there break up into terminal ramifications, is evidently associated functionally with the nervus conarii, for the fibres of the latter run in close relation with their branches. In the human brain no macroscopically visible branches of the chorioidal arteries have such an intradural extension. On the other hand, a microscopic arteriole follows the nervus conarii of Man into the floor of the straight sinus, and a fine plexus of blood-vessels can also be followed up in the tunica adventitia of the vena magna cerebri.

The existence, in relation to the opening of the great vein of Galen into the straight sinus, of an arachnoidal formation filled with blood sinusoids, and with an intrinsic structure recalling that of erectile tissue, is an observation which has not apparently been made before in the human brain[1]. It has been pointed out that this peculiar formation (here termed the suprapineal arachnoid body) possibly constitutes a neurovascular mechanism whereby the venous return through the great vein of Galen is regulated. The pial vessels in the neighbourhood of the suprapineal body are innervated by abundant nerve fibres, and the latter could supply the means whereby the filling of the blood sinuses is controlled. It has been shown that the nervus conarii in its course to the floor of the straight sinus traverses the cavity of one of the blood sinuses within the suprapineal body, but the material has shown no evidence that any of the fibres of this nerve end here. It may be noted that when the brain is removed in the ordinary way from the intracranial cavity the suprapineal arachnoid body will be torn through at its attachment to the dura mater of the tentorium cerebelli; consequently its real nature will escape detection in such material. Those who are accustomed to the examination of human brains in anatomical or pathological departments, however, will be familiar with the small patch of dense and opaque pia-arachnoid tissue which is always to be seen on the superior vermis of the cerebellum where this lies in relation to the anterior end of the

[1] This structure has been observed histologically in four specimens. It is perhaps hardly necessary to say that its constancy can only be determined by the examination of many more brains.

straight sinus. This patch marks the position of the suprapineal arachnoid body, and there are frequently found in relation to it a few small arachnoid granulations of the usual type.

In the monkey a suprapineal body comparable to that of the human brain has not been identified. On the other hand, recurrent twigs from the artery accompanying the nervus conarii form a rich plexus in the pial tissue on the ventral surface of the great vein of Galen, and it is possible that these form part of a neurovascular mechanism which may play a role similar to that suggested for the suprapineal body. In this connexion it may be recalled that among the vessels in this pial tissue some sections show a globular skein of nerve fibres resembling a sensory nerve ending. It is also possible that the terminal branches of the chorioidal artery, which ramify in a curiously tortuous manner in the wall of the anterior end of the straight sinus (and which are accompanied by fibres of the nervus conarii), may be related to a reflex mechanism concerned with the venous return through the vein of Galen. These are problems which now require investigation.

SUMMARY

1. The pineal gland of the rhesus macaque contains a central core of neuropil in which are embedded numerous large ganglion cells with branching processes.

2. Contributing to this network of nerve fibres are perivascular plexuses accompanying branches of the chorioidal arteries which penetrate the pineal gland from its lateral aspect, and also a conspicuous fasciculus which can be traced to the apex of the pineal gland where it emerges as the nervus conarii.

3. Fibres from the habenular and posterior commissures enter the substance of the pineal gland through its peduncle, but on tracing these through serial sections many are found to be merely aberrant commissural fibres which enter the peduncle on one side and leave it on the other. It is considered not improbable that all the fibres from the commissures which have been followed into the parenchyma of the pineal gland may be of a similar nature.

4. Some of the fibres of the posterior commissure certainly terminate in relation to the ependymal epithelium lining the posterior wall of the pineal recess, and here show regular beading and minute end-bulbs.

5. The central core of neuropil in the macaque epiphysis is pervaded by a rich capillary plexus. Elsewhere the gland shows no great vascularity.

6. No system of blood-vessels is present which links the pineal gland with cell collections in the epithalamus comparable to that which has been described in relation to the hypophysis and the hypothalamus.

7. In the monkey a chorioidal vessel extends up into the wall of the straight sinus, where it ramifies in a subendothelial position. From this vessel recurrent twigs descend to enter the pineal gland at its apex.

8. The nervus conarii in the monkey has been traced from the central core of neuropil in the pineal gland into the wall of the straight sinus. Here it

ramifies in a plexiform manner in a subendothelial position, its fasciculi usually bearing a close topographical relation to branches of the chorioidal artery which are also found here.

9. Some sections show a globular skein of nerve fibres in the pial tissue between the pineal gland and the straight sinus, resembling a sensory nerve-ending.

10. In the human pineal gland the nervus conarii has been recognized. It emerges from the tip of the gland and runs an uninterrupted and apparently unbranched course to reach the dura mater of the tentorium cerebelli. Here it runs back in the floor of the straight sinus occupying a subendothelial position. The nerve has been followed in serial sections for a distance of 20 mm. Its destination (in a peripheral direction) has not been determined.

11. A formation of the arachnoid has been described in relation to the floor of the straight sinus where the great vein of Galen opens into it. This structure somewhat resembles a large arachnoid granulation of the usual type, but its stroma consists of dense pial tissue containing a sinusoidal plexus of blood vessels and several large blood sinuses. It has been termed the suprapineal arachnoid body.

12. From its structure and disposition it is suggested that the suprapineal body may provide a ball-valve mechanism whereby the venous return from the great vein of Galen is regulated and controlled.

13. On its way to the dural floor of the straight sinus the nervus conarii in the human brain was found in two specimens to pass through the suprapineal body and traverse one of the blood sinuses therein.

14. No evidence was found, either in macaque or human material, for the existence of the "ganglion conarii" as described by Pastori.

I wish to acknowledge the valuable assistance of my technician, Mr E. A. Thompson, in the preparation of the histological material and particularly in completing the beautiful series of sections through the human pineal gland and adjacent tissues of which Text-fig. 7 represents one section. I would also thank Mr W. Chesterman for the examples of his skill in photomicrography.

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EXPLANATION OF PLATES I AND II

PLATE I

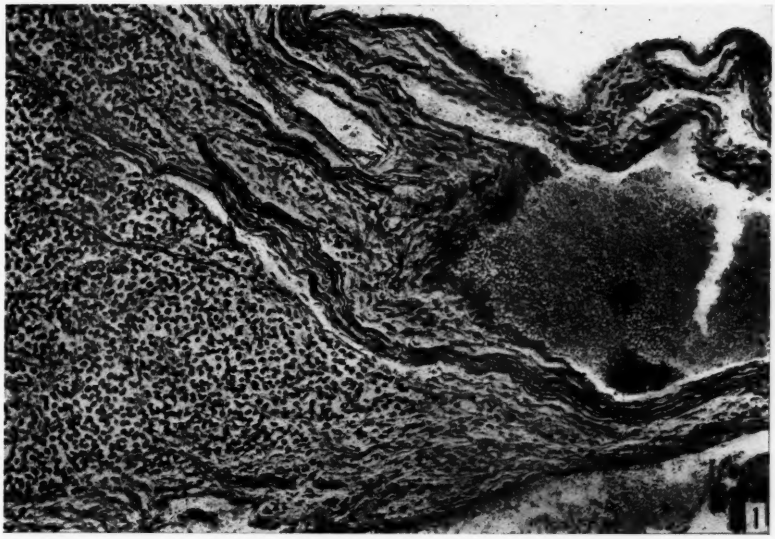
Fig. 1. Sagittal section of the tip of the pineal gland of a rhesus macaque, stained by Bodian's protargol technique. The nervus conarii is seen emerging from the gland and passing along the ventral wall of the vena magna cerebri. ×118.

Fig. 2. Section of the pineal gland of the rhesus macaque, showing the central core of neuropil and nerve-cells. Stained by Bodian's protargol method. ×160.

PLATE II

Fig. 3. Section of the pineal gland of the rhesus macaque, showing two ganglion cells. Stained by Bodian's protargol method. ×500.

Fig. 4. Section through the suprapineal arachnoid body of a human brain, stained by Bodian's protargol method. The body is filled with sinusoidal blood-vessels containing blood corpuscles. In the dura mater surrounding the body is seen the nervus conarii, cut in two portions (*n.n.*). Above is seen a lobule of the suprapineal body which in this section has the appearance of being detached from the main part.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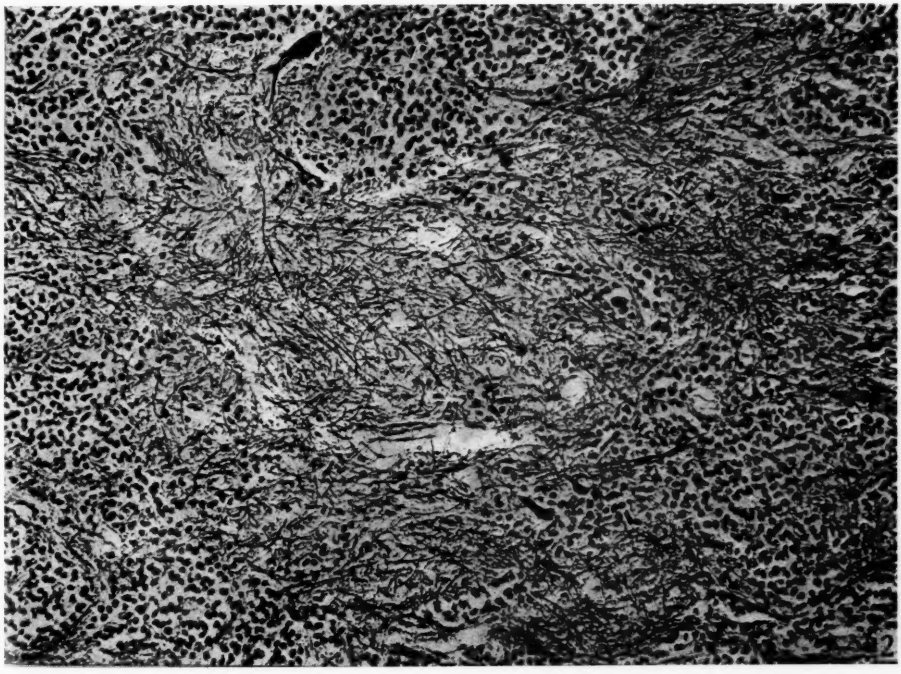
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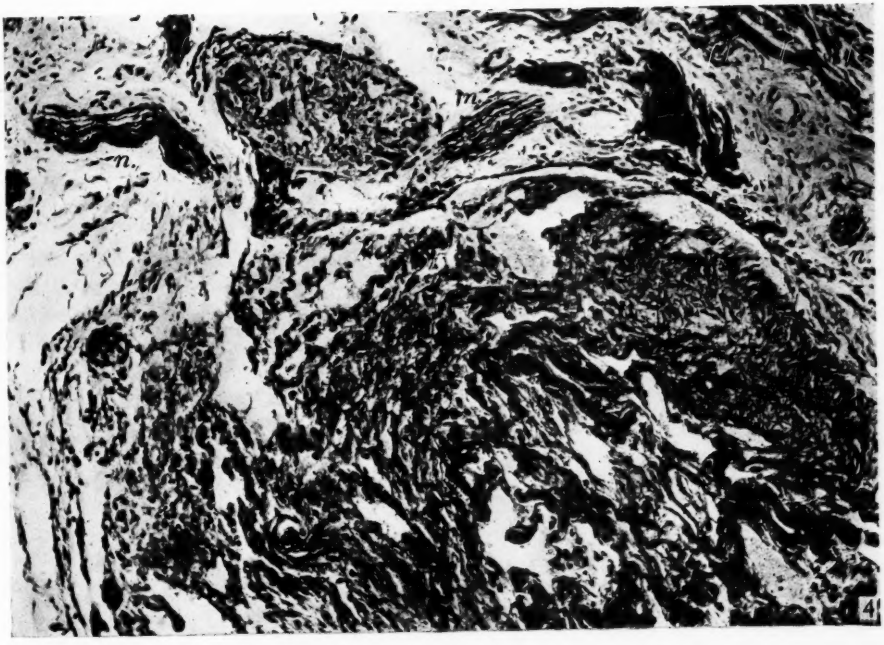

LE GROS CLARK—THE PINEAL GLAND

UNIV. OF MICH.

THE PARANASAL SINUSES OF THE ANTHROPOID APES

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INTRODUCTION

From time to time various writers have given some account of the anatomy of the nasal fossae and associated paranasal sinuses of anthropoid apes. Thus Seydel (1891) has given an excellent account of the structure of the nasal fossa in Primates, while Paulli (1899, 1900) has not only provided a clear (though unillustrated) account of the primate arrangement of parts, but has also worked out in considerable detail its morphology in subprimate mammals. Between them, these two authors have presented a tolerably complete picture of the form and evolution of the paranasal sinuses, from their initial appearance in eutherian phylogeny to their greatest elaboration in various extant specialized forms. Nevertheless, in their study of anthropoid apes both authorities were seriously handicapped by scarcity of material, particularly of the gorilla and chimpanzee, and it is impossible from their descriptions, accurate as they are, easily to comprehend the interrelationships of the various structures present in the anthropoid ape group.

The gaps in our knowledge have been partially filled by Keith (1902), Bolk (1917), Weidenreich (1924), Wegner (1936), Wood Jones (1938), and others, but none of these later authors has attempted any re-assessment of the anthropoid ape types as a whole, though the possible phylogenetic significance of the paranasal sinuses has been not infrequently suggested. Further, since for no anthropoid ape are figures or descriptions available showing all the sinuses in their detailed relationships to individual cranial bones, it becomes necessary first to give a short account of these structures in each genus of anthropoid apes, and only thereafter to attempt the institution of comparisons between them or any appreciation of their phylogenetic history.

Herein the ordinary terminology of the conchae as employed in comparative anatomy is adopted, i.e. nasoturbinal, maxilloturbinal and ethmoturbinals I, II and III, corresponding respectively to the agger nasi, inferior concha, and the middle, superior and supreme conchae of man. The terms "inferior", "middle" and "superior meatus" are applied in their human connotation to lower Primates. Though anatomical and clinical custom has sanctioned the application of the term "processus uncinatus" both to the

ethmoidal bony process itself and to the ridge of mucous membrane which covers it, we have here preferred Peter's (1902) term "plica semilunaris" for the mucous ridge alone. "Bulla ethmoidalis", though not ideally descriptive of the homologous structure in anthropoid apes, has been retained to designate both the relevant mucosal elevation and the underlying osseous formation. "Hiatus semilunaris" is used (in Zuckerkandl's sense) as meaning the gap between the bulla ethmoidalis and the plica semilunaris, and as excluding any cavity to which that gap may lead (Layton, 1934). The cavity in the lateral nasal fossa wall into which the hiatus semilunaris leads is called by His (1895) and Schaeffer (1910 *a*) the "infundibulum ethmoidale", but, as Layton has pointed out, this term has been too indiscriminately employed in the past to have retained any definite meaning, and we have therefore preferred instead a latinization of Layton's own term "semilunar groove", namely "sulcus semilunaris". (Admittedly "semilunar" is not strictly descriptive of certain of the several varieties of form which the sulcus may assume: nevertheless the term is retained here partly because it is already established in nasal anatomy and partly to avoid the pedantry inseparable from the adoption of a more precisely descriptive label.)

Further, in human descriptive anatomy, the term "concha nasalis inferior" is applied both to an intranasal elevation of mucous membrane and to the underlying, causative skeletal element, and with some justice, since in man the two are approximately co-extensive. In the anthropoid ape nasal fossa, however, matters are otherwise, the equivalent of the processus lachrymalis et ethmoidalis of the human skull taking the form of a large vertical bony plate (pars verticalis) in the lateral nasal wall. Hence the distinction is herein made between the skeletal element itself ("inferior turbinate") and the less extensive conchal elevation ("maxillo-turbinal").

THE GIBBONS AND THE PRIMITIVE ANTHROPOID APE TYPE

The structure of the gibbon nasal fossa is variable: of two specimens discussed here in detail, one manifests relatively elaborate nasal structures, the other a more simplified arrangement.

In the first specimen, *Hylobates lar leuciscus* (R.C.S. 30 A) the intact but rather shrunken mucous membrane reveals the structures in the middle meatus particularly well without need of conchal disturbance. The nasoturbinal, maxilloturbinal and ethmoturbinals I, II and III, are well-developed; the smooth-walled inferior meatus receives the nasolachrymal duct: the wide middle meatus bears on its lateral wall two ridges (*a*) the plica semilunaris, formed by the mucous membrane overlying the processus uncinatus of the ethmoid, and above this, (*b*) the bulla ethmoidalis. Between the two the semilunar gap (hiatus semilunaris) leads to the shallow semilunar groove (sulcus semilunaris), in the floor of which, at its posterior part, lies the opening (ostium maxillare) of the maxillary sinus. Thus the sinus opens, not directly

but indirectly, into the middle meatus, by way of the semilunar groove and semilunar gap. Posteriorly a large sphenoidal sinus is present but ethmoidal and frontal sinuses are wanting.

A second specimen of the same species (R.C.S. 30 B) reveals on examination a precisely similar condition of parts.

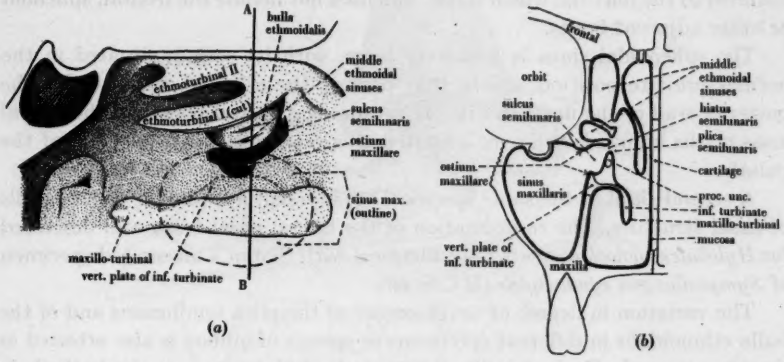


Fig. 1. Schemata of human lateral nasal fossa wall to illustrate structure and the terminology employed: (*a*) surface view, ethmoturbinal I (concha media) cut to expose bulla ethmoidalis, etc., (*b*) vertical section along A–B.

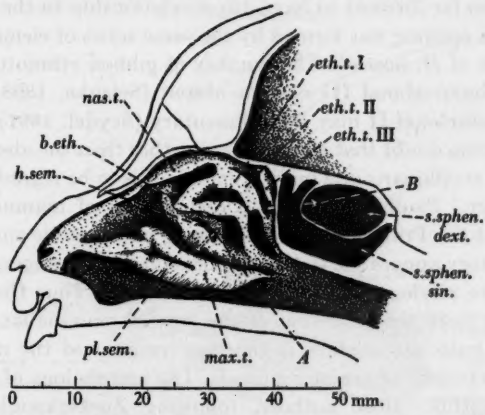


Fig. 2. Lateral nasal fossa wall in *Hylobates lar leuciscus* (R.C.S. 30 A), wet specimen. Arrow *A* passes through hiatus semilunaris and sulcus semilunaris into maxillary sinus: B enters sphenoidal sinus.

In a macerated skull of a different species, *H. hoolock* (R.C.S. D 1), the main turbinals are developed as before, though in different relative proportions. The middle meatus, again wide but here quite smooth-walled, presents neither bulla ethmoidalis nor uncinate process, so that in the fresh state a plica semilunaris was presumably wanting. In this specimen therefore the maxillary

sinus opens directly into the middle meatus without the intervention of a sulcus and hiatus semilunaris. The meatal lateral wall is constituted by the maxilla, the nasal surface of the lachrymal, the large vertical plate of the inferior turbinate, and a small part of the ethmoid, all of which participate in the formation of the bony opening into the maxillary sinus. The sinus itself is confined to the maxilla, which it fills, and does not invade the frontal, sphenoid or other adjacent bones.

The sphenoidal sinus is relatively large, with its ostium situated in the normal primate position, i.e. in that part of the ethmoid which forms the posterior wall of the nasal cavity. It invades the frontal bone and the lateral mass of the ethmoid, and over a limited area reaches the vertical plate of the palatine.

A second skull of the same species (D 2) is essentially similar in its details of nasal structure. The conformation of the lateral nasal fossa wall described for *Hylobates hoolock* appears to be identical with that of a macerated specimen of *Symphalangus syndactylus* (R.C.S. 60).

The variation in degree of development of the plica semilunaris and of the bulla ethmoidalis in different specimens or species of gibbon is also attested in the literature. In Zuckerkandl's (1887) skull of *Hylobates concolor* both these structures were well-developed, but in Seydel's (1891) wet specimen, *Hylobates* sp., although the bulla and plica were present in rudimentary fashion, they were situated too far forward to have direct relationship to the ostium maxillare, so that this opening was formed by the same series of elements as obtains in our specimen of *H. hoolock*. The number of gibbon ethmoturbinals is also variable, for ethmoturbinal III may be absent (Selenka, 1898; Paulli, 1900) and even ethmoturbinal II may be rudimentary (Seydel, 1891).

There can be no doubt that the presence, rather than the absence, of a well-developed plica semilunaris and bulla ethmoidalis is to be regarded as the more primitive feature. Paulli's (1900) extended studies of mammalian material have shown that the Primates as a whole, being microsmatic animals, manifest a reduced olfactory apparatus, so that a more complex arrangement is likely to have given place phylogenetically to a more simple. Thus the three ethmoturbinals result from the reduction of the typical mammalian set of four or more, and the bulla ethmoidalis is the last remnant of the numerous ectoturbinals characteristic of osmatic animals. The morphology of the plica semilunaris is doubtful. Most authors, following Zuckerkandl (1887), have interpreted it as a part of the nasoturbinal, but Peter (1902) has claimed that it develops as an independent structure. It is clear, at least, that it forms part of the primitive primate inheritance, since Seydel (1891) has shown that it is already developed in platyrrhine and catarrhine monkeys. Hence in the possession of a plica semilunaris separated by a hiatus semilunaris from an ethmoidal bulla, and of a maxillary sinus opening into a sulcus semilunaris, the first gibbon described above (R.C.S. 30A) is to be regarded as primitive. It is possible that the earliest anthropoid apes possessed a still more complex nasal

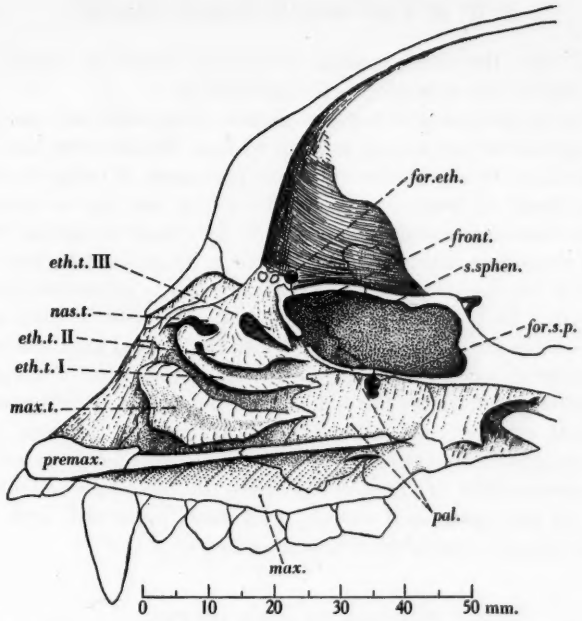


Fig. 3. Lateral nasal fossa wall of *Hylobates hoolock* (R.C.S. D 1), macerated specimen, undissected.

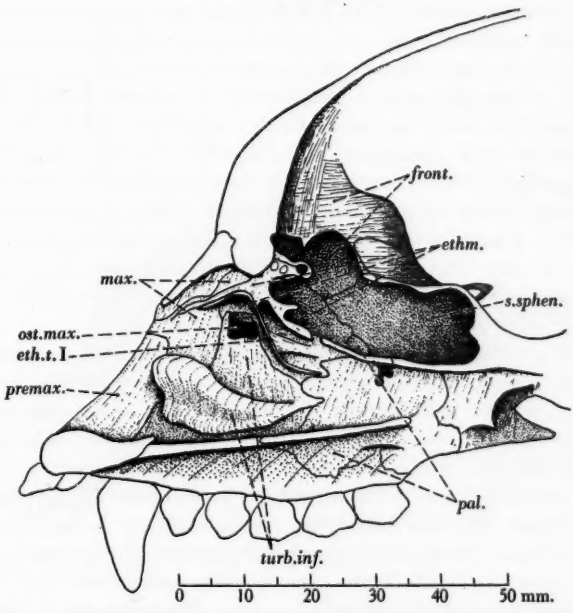


Fig. 4. Same specimen as preceding, with ethmoturbinal I removed and sphenoidal sinus fully opened.

cavity, and that the extra conchae sometimes found in human embryos (Schaeffer, 1910 *b*) are of phylogenetic significance.

Again, in its possession of but two sinuses, sphenoidal and maxillary, the gibbon is a primitive type among anthropoid apes. Paulli (1900) has shown the maxillary sinus to be a primitive eutherian possession, it being the only paranasal sinus found in Insectivora and Chiroptera, and its occurrence being constant in other mammalian orders, save in very small or aquatic forms. The sphenoidal sinus also, although not so ancient an acquisition nor so widely distributed as the maxillary sinus, is, nevertheless, a primitive primate possession (Seydel, 1891). On the other hand the true frontal (*vide infra*) and ethmoidal sinuses are confined to the African anthropoid apes and man. Thus the sole specialization of importance in the nasal apparatus of the gibbon lies in the relatively large size of the sphenoidal sinus and in the invasion thereby of the frontal, ethmoid and palatine bones. Though it is possible, as Pocock (1927) has suggested, that the gibbons represent a secondarily dwarfed type of ape, such consideration need not detract from the conclusion here drawn that, in respect of their paranasal sinuses, they have preserved, with but little change, the primitive anthropoid ape inheritance.

SINUSES OF THE ORANG

In a wet specimen (R.C.S. 990) of a young orang, wherein all the maxillary milk teeth, save the canine, have erupted, the nasal fossa displays but two well-developed conchae, viz. the maxilloturbinal and ethmoturbinal I, although an ill-developed nasoturbinal and ethmoturbinal II are also recognizable. The sphenoidal sinus is represented by a small dimple only in the posterior nasal fossa wall, but the capacious maxillary sinus seems already to have attained its adult disposition. No trace exists of any plica semilunaris, nor, consequently, of either hiatus or sulcus semilunaris; the maxillary sinus therefore opens by an ostium directly (though relatively far back) into the middle meatus. Conditions are essentially similar in a second, somewhat larger and older specimen (R.C.S. E 166) wherein almost the entire permanent dentition has been acquired, and an identical type of maxillary ostium is present in an adult male specimen (R.C.S. E 167 *a*).

A skull of a young adult male orang (St. Thos. 3) has the paranasal sinuses already fully developed, although the cranial sutures, save only the maxillopremaxillary, persist with perfect distinctness. The sphenoidal sinuses are relatively very small, occupying merely the most inferior part of the sphenoidal body: right and left sinuses, separated by an osseous septum, are equally developed, and each opens in the customary primate site on the posterior wall of the nasal fossa. The maxillary sinus, on the contrary, is enormous, and has secondarily invaded every bone (save the premaxilla) which it may ever invade in any mammal (cf. Paulli, 1900). Its opening, again direct into the middle meatus without trace of any uncinate process, is bounded above and anteriorly

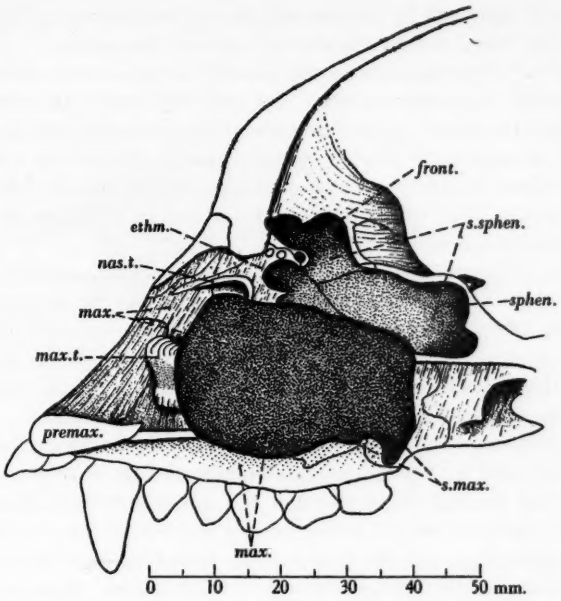


Fig. 5. Same specimen as in Fig. 4 with maxillary sinus fully opened.

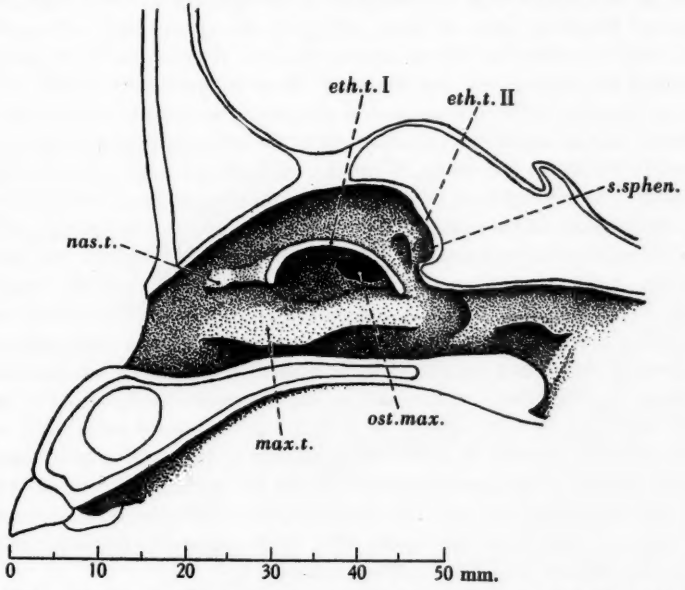


Fig. 6. Lateral nasal fossa wall of young orang (R.C.S. 990), wet specimen, with ethmoturbinal I cut to expose ostium maxillare.

by the ethmoid, inferiorly by the vertical plate of the inferior turbinate, whilst posteriorly it is completed by the vertical plate of the palatine.

The sinus not only occupies the entire maxilla but also invades the lachrymal, frontal, ethmoid, zygomatic, palatine and sphenoid bones. Its sphenoidal extension, situated dorsal to the true sphenoidal sinus, invades both the pterygoid process and the zygomatic process of the temporal. The canals for the infra-orbital and ethmoidal neuro-vascular structures project into the sinus, as does the nasolachrymal duct, whilst the optic nerve and the internal carotid artery encroach upon its sphenoidal extension.

These orang specimens would appear to be typical in respect of the constitution of their nasal fossae. Ethmoturbinal II may be altogether absent (Zuckerkandl, 1887; Seydel, 1891), whilst one or other sphenoidal sinus may be suppressed or represented by a shallow sphenoidal depression only (Paulli, 1900). The third specimen described above appears to be the first recorded showing equal development of right and left sphenoidal sinuses.

The true nature of the sphenoidal extension of the orang's maxillary sinus was first recognized by Seydel (1891) who corrected the earlier and mistaken impression that this cavity represented the sphenoidal sinus proper. Later, Paulli (1900) confirmed Seydel's observations and conclusions, but the work of both these authorities appears to have been forgotten, as Underwood (1910) again misunderstood the precise anatomy of these sinuses. More curious still is the statement by Wegner (1936) that in the young orang of milk dentition age a group of ethmoidal cells lies anterior to the sphenoidal sinus, and that a subsequent breaking down of these cells puts the sphenoidal and maxillary sinuses into secondary but direct communication. Wegner, however, provided no detailed description nor any figure of these supposed ethmoidal sinuses, but as in Seydel's (1891) animal and in our young specimens no trace of such was found, and as, moreover, the anatomy of the true sphenoidal sinuses is now adequately known in the orang, Wegner's explanatory notion may be finally dismissed. It is evident from Seydel's figures that his specimen resembled ours in the constitution of the orifice of the maxillary sinus, but he interpreted as a processus uncinatus that margin of the ethmoid which meets the inferior turbinate. Such an interpretation, however, appears untenable, since the typical uncinate process invariably stands well away from the lateral meatal wall, raising a distinct ridge of mucous membrane (the plica semilunaris) and thus causing the ostium maxillare to lie buried in the sulcus semilunaris. In the orang, on the contrary, the ostium maxillare lies flush with the lateral meatal wall, and in view of this, as well as of the known variability of the gibbon uncinate process, it is not unreasonable to infer, as is here done, the complete absence of an uncinate process from the orang skull. Again Weinert (1926) has described both anterior and posterior ethmoidal cells in the orang skull, but it is clear from his figures that these supposed ethmoidal cells are merely outlying parts of the maxillary sinus.

The frontal extension of the maxillary sinus, first described by Seydel (1891)

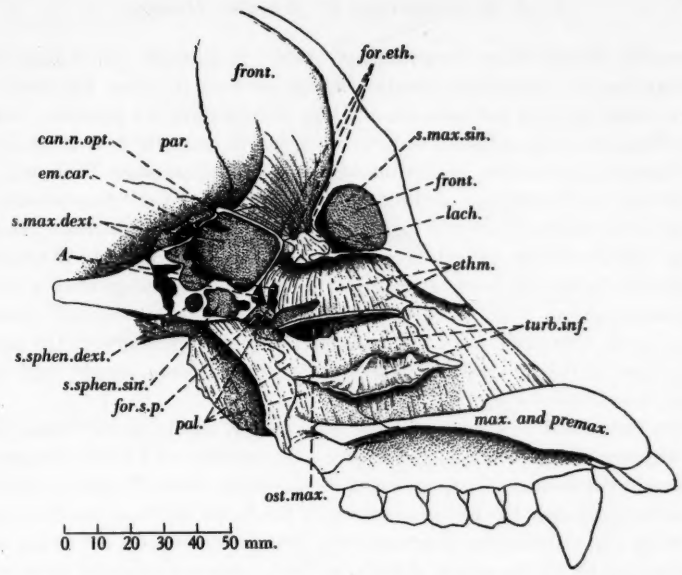


Fig. 7. Lateral nasal fossa wall of adult orang (St Thos. 3) with ethmoturbinal I cut to expose ostium maxillare. A = hiatus between ethmoid and palatine, filled in life by mucous membrane, separating maxillary sinus from nasal cavity.

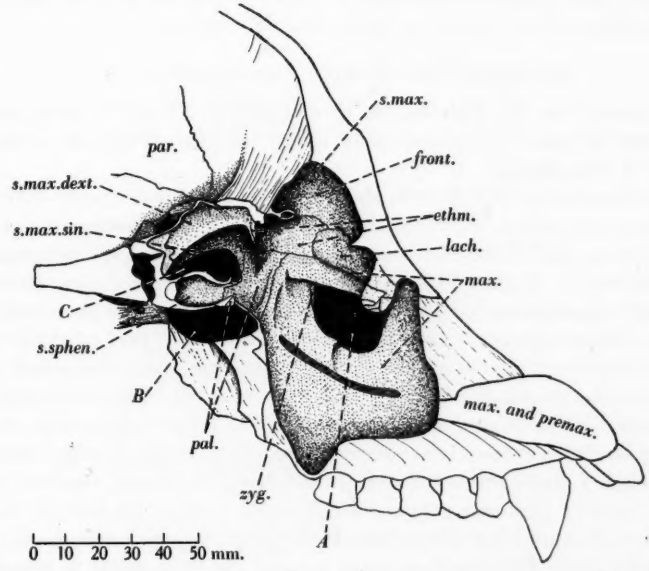


Fig. 8. Same specimen as in Fig. 7 with maxillary and sphenoidal sinuses fully opened. A = extension of maxillary sinus into zygomatic, B = extension into pterygoid process, C = extension into sphenoidal great wing and into temporal.

and recently discussed by Bauermeister (1939), is unusual, yet it may assist understanding of apparently similar frontal cavities in other Primates. As Selenka (1899) pointed out, absence of a true frontal sinus is a primitive feature among Primates. This indeed is only to be expected, since the true frontal sinus is of ethmoidal derivation, and ethmoidal sinuses are, amongst Primates, confined strictly to the African anthropoid apes and man. Yet "frontal sinuses" have been described in several genera, including *Lemur*, *Nycticebus*, *Alouatta* (Seydel, 1891), *Cebus* (Weidenreich, 1924) and *Daubentonia*, *Hapalemur*, *Propithecus*, *Indris* (Weinert, 1926). But as Paulli (1900) suggested, a critical examination shows that in each instance the so-called "frontal" sinus is atypical in its connexion with the nasal cavity, never opening into the antero-superior part of the middle meatus as the true frontal sinus should, and therefore not homologous with that structure.

From the primitive anthropoid ape type already outlined, the orang differs in the absence of any processus uncinatus and therefore of a semilunar groove, as well as in the enormous expansion of its maxillary sinus. Whilst in the other large anthropoid apes the pneumatization of the facial skeleton has been partly effected by the elaboration of neomorphic ethmoidal cells, in the orang it has been achieved by an expansion of the maxillary sinus so exuberant as to reduce the sphenoidal sinuses to insignificance.

It seems therefore most probable that the orang diverged from the anthropoid phylogenetic stem before the appearance therein of ethmoidal sinuses, and that it met the functional demands for a lightening of the skull by a structural specialization of its primitive primate sinus inheritance.

SINUSES OF THE CHIMPANZEE

As, apart from the gibbons, the chimpanzee manifests the most primitive disposition of paranasal sinuses seen in any anthropoid ape, it is here considered in some detail.

A wet specimen (R.C.S. 898) shows a maxilloturbinal doubly-rolled as in the gibbon and orang, and overhanging a deep inferior meatus which receives a non-dilated naso-lachrymal duct. A small nasoturbinal is continuous with ethmoturbinal I. In the middle meatal wall a slightly everted plica semilunaris, separated by a distinct hiatus semilunaris from a narrow but prominent bulla, leads to a deep sulcus semilunaris, in the antero-inferior part of which appears the constricted ostium maxillare. From the anterior part of the sulcus another opening leads into a small, spherical anterior ethmoidal sinus, which bulges into the interior of the frontal sinus. The frontal sinus itself opens into the angle between the bulla ethmoidalis inferiorly and the attachment of ethmoturbinal superiorly. A small ethmoturbinal II overhangs a narrow superior meatus, whence opens a posterior ethmoidal cell which reaches the wall of the orbit. A distinct ethmoturbinal III occurs. Posteriorly a narrow orifice leads into the sphenoidal sinus. The maxillary sinus invades the hard palate to become contiguous with its fellow of the opposite side though separated therefrom by a com-

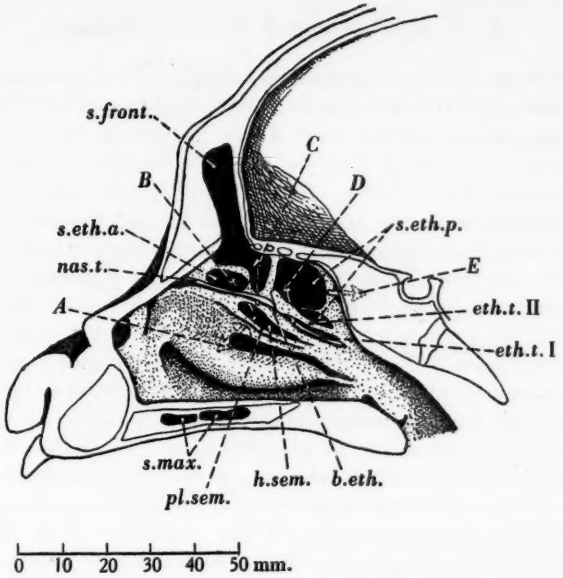


Fig. 9. Lateral nasal fossa wall of chimpanzee (R.C.S. 898), wet specimen, with ethmoturbinal I removed and with frontal and ethmoidal sinuses opened. Arrow A passes from sulcus semilunaris through ostium maxillare (broken line) into maxillary sinus; B from sulcus semilunaris into anterior ethmoidal sinus; C from suprabullar groove into frontal sinus; D from superior meatus into posterior ethmoidal sinus; E enters sphenoidal sinus.

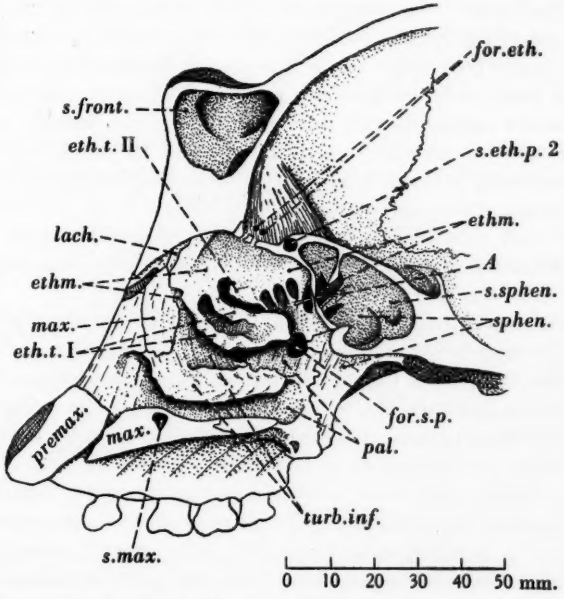


Fig. 10. Lateral nasal fossa wall of chimpanzee (R.C.S. 12.53), macerated specimen: arrow A enters sphenoidal sinus.

plete bony septum. (Such an intrapalatal extension of the chimpanzee antrum was by Underwood (1910) rather unhappily termed "supra-palatine".)

The correspondence between these several features and the constitution of the underlying osseous nasal fossa wall is well exemplified by the skull of an adult chimpanzee (R.C.S. 12.53). Herein a doubly-rolled maxilloturbinal is again encountered, while the uncinate process present has that everted disposition and form which characterizes the plica semilunaris of the wet specimen. Except where attached at its anterior extremity to the ethmoidal lateral mass, the processus uncinatus remains free from articulation with other bones, so that, although when viewed medially it overlaps both the ethmoid and the palatine vertical plate, actually it stands well away from both these structures. Thus in the macerated preparation, between the inferior border of the processus uncinatus and the superior margin of the inferior turbinate, there appears a gap or fontanelle, closed, in life, by the soft tissues forming the medial wall of the sulcus semilunaris. As the processus uncinatus terminates freely, the hiatus semilunaris in the macerated skull remains unclosed posteriorly. A bulla ethmoidalis being indistinguishable in this particular skull, the superior boundary of the hiatus semilunaris is formed instead by the attachment of ethmoturbinal I. A frontal sinus opens into the angle between ethmoturbinal I and the middle meatal lateral wall, immediately antero-superior to the hiatus; in the absence of an ethmoidal bulla its precise morphological position is indefinable, although probably identical with that of the frontal sinus in the wet specimen (No. 898). The sinus itself extends from the ethmoid into the frontal and lachrymal. An anterior ethmoidal sinus, opening into the sulcus semilunaris, lies chiefly within the lachrymal, but is roofed to a small extent by the frontal. In the floor of the sulcus semilunaris the maxillary orifice (ostium maxillare) is completely circumscribed—by the ethmoid superiorly, by the inferior turbinate anteriorly and inferiorly, and by the palatine posteriorly. The sinus extends considerably into the hard palate, including its premaxillary element. Posteriorly it invades the ethmoid so as to effect apposition with the sphenoidal sinus. Two posterior ethmoidal sinuses open into the superior meatus and each attains the orbital wall: the sphenoidal sinus, occupying partly the sphenoid and partly the ethmoid, has extensions into both the alisphenoid and pterygoid processes.

In a second macerated specimen (C 5) the uncinate process effects a minute articulation (1 mm. in extent) with a narrow ascending process from the inferior turbinate: otherwise the nasal fossa wall agrees in osseous constitution with that seen in specimen R.C.S. 12.53 and in other crania examined.

Accounts of the structure of the chimpanzee nasal fossa are so scarce and so incomplete that it is impossible to determine whether the present specimens agree with examples previously recorded in the literature. Sonntag (1923), dissecting the nasal fossa of a young female chimpanzee, encountered, but did not identify, "a movable mucosa-covered bony crest"—clearly, from his description, the uncinate process underlying the plica semilunaris. Selenka

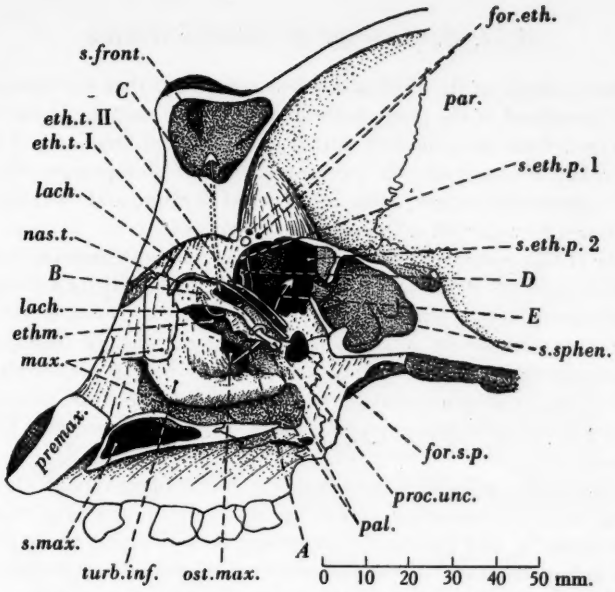


Fig. 11. Same specimen as preceding with ethmoturbinals I and II removed, intrapalatal extension of maxillary sinus and posterior ethmoidal sinuses opened. Arrow A curves over uncinate process and enters ostium maxillare; B passes from sulcus semilunaris into anterior ethmoidal sinus; C enters frontal sinus; D and E pass from superior meatus into posterior ethmoidal sinuses.

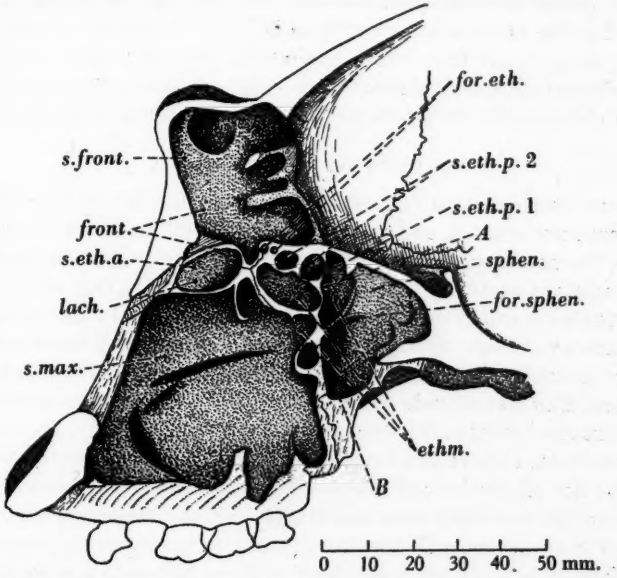


Fig. 12. Same specimen as preceding, dissected to plane of medial orbital wall and with maxillary, frontal and sphenoidal sinuses opened. A = extension of sphenoidal sinus into great wing; B = extension into pterygoid process.

(1898), from a study of the skull as a whole, suggested that the chimpanzee is the least specialized of the great anthropoid apes; he considered the orang to have diverged from the primitive anthropoid ape stem-form and to have become therefore less man-like in structure than the chimpanzee, whose pronounced supraorbital ridges, wide interorbital septum and development of frontal sinuses he regarded as generic specializations.

Wegner (1936) considered the chimpanzee as the most man-like of anthropoid apes in respect of its nasal fossa constitution, instancing its well-developed ethmoidal sinuses and its long sulcus semilunaris. As, however, he erroneously supposed ethmoidal sinuses to be present in young orangs, he failed to appreciate the significance of the first appearance of such structures in the anthropoid group. Further, he over-estimated the profusion of ethmoidal sinuses in the chimpanzee, wherein (as also commonly in the gorilla) no more than three such cells appear.

The chimpanzee resembles the more primitive gibbon type in the number and arrangement of its conchae, and in the anatomical relations of its plica and sulcus semilunaris, and (when this structure is developed) of its ethmoidal bulla. It differs, however, in the possession of ethmoidal and frontal sinuses, and possibly also in a certain incompleteness of ossification of the medial wall of the sulcus semilunaris which is responsible for fontanelle formation. Though such characters represent a specialization and an advance upon the gibbon type, they obtain also in the gorilla and in man, and hence would appear to be relatively primitive anthropoid features. The sole specific specialization encountered in the chimpanzee nasal fossa is the intra-palatal extension of the maxillary sinus, apart from which this animal appears to have retained that structural nasal equipment characteristic of the common anthropoid ape stock after the phylogenetic deviation therefrom of the gibbons and the orang.

SINUSES OF THE GORILLA

In a wet specimen of a four years old gorilla (L.G.A.) all four permanent first molars have erupted, and the permanent central incisors are in process of eruption; the nasal mucosa is intact and the paranasal sinuses appear already to have attained maturity of arrangement and extent. The inferior meatus receives the lower end of the dilated nasolachrymal duct. In the middle meatal wall a plica semilunaris appears as an everted ridge, and a bulla ethmoidalis as a short elevation above it, the two being separated by an intervening hiatus semilunaris. This last extends posteriorly beyond the relatively short plica and bulla, so that its hinder part is bounded by two structures only, the attachment of ethmoturbinal I above and the middle meatal lateral wall behind and below. In the absence of any distinct sulcus semilunaris, the hiatus semilunaris leads directly into the maxillary sinus and into the anterior ethmoidal sinus. A well-developed frontal sinus, with floor invaginated by the anterior ethmoidal sinus, opens inferiorly between the attachment of ethmoturbinal I and the ethmoidal bulla. Of the two posterior ethmoidal sinuses opening into the superior meatus,

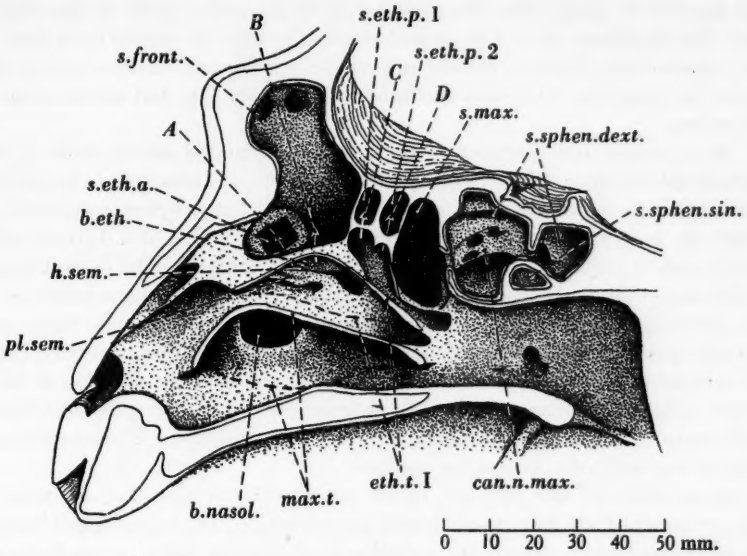


Fig. 13. Lateral nasal fossa wall of young gorilla (L.G.A.), wet specimen, with maxillo-turbinal and ethmoturbinal I removed and with frontal and ethmoidal sinuses opened. Arrow A passes from hiatus semilunaris to anterior ethmoidal sinus; B from suprabullar groove to frontal sinus; C and D from superior meatus to posterior ethmoidal sinuses.

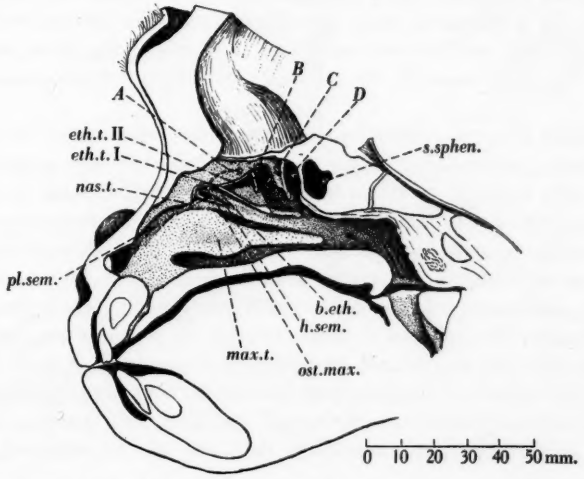


Fig. 14. Lateral nasal fossa wall of young gorilla (T.C.D.), wet specimen, with ethmoturbinals I and II removed. Arrow A passes from suprabullar groove to frontal sinus; B and C from superior meatus to posterior ethmoidal sinuses; D through ostium of sphenoidal sinus.

the anterior is shallow but the posterior is deeper and extends to the orbital wall. The maxillary sinus is large and attains the anterior cranial fossa floor in two places—first, between frontal and posterior ethmoidal sinuses, second, between the posterior ethmoidal and sphenoidal sinuses. The last named sinus is capacious.

In a younger wet specimen (T.C.D.), wherein the first molars alone of the permanent dentition have erupted, the nasal mucosa is intact and the general anatomy of the nasal fossa is much the same, save that the plica semilunaris is relatively longer. The plica extends posterior to the hiatus as a distinct ridge which ends by fusing with the main mass of the ethmoid. The frontal sinus, which superiorly has not yet gained the supraorbital region, opens below (as in the previous specimen) between ethmoturbinal I and the ethmoidal bulla, and extends posteriorly into the ethmoidal region. Two posterior ethmoidal cells, of unequal size, are recognizable, opening into the superior meatus. A half-grown sphenoidal sinus occupies the anterior portion of the sphenoidal body and opens into the spheno-ethmoidal recess. A well-dilated nasolachrymal duct is received into the inferior meatus.

In a third, and still younger, intact wet gorilla specimen (L.G.B.) none of the permanent teeth have as yet begun their eruption. Of the paranasal sinuses there are present the maxillary, a rudiment of the sphenoidal, a single, large, posterior ethmoidal, and an immature frontal which has not yet invaded the frontal bone. The nasoturbinal, maxilloturbinal and ethmoturbinals I, II and III are all characteristically developed. The inferior meatus receives the already dilated nasolachrymal duct. The middle meatus presents on its lateral wall a long and prominent plica semilunaris, separated from the bulla ethmoidalis above by a relatively deep sulcus semilunaris, in the anterior part of which appears the oval ostium maxillare. Posteriorly the plica semilunaris fuses with the main mass of the ethmoid. No distinct fronto-nasal duct is detectable.

A condition of parts essentially similar to that obtaining in the foregoing wet specimens, particularly in the matter of number and appearance of ethmoidal cells, is displayed by an infant gorilla skull (R.C.S. 30 A) which has its milk dentition *in situ*, and its first permanent molars far from eruption. The inferior meatus receives an already well-dilated nasolachrymal duct; the maxillary sinus occupies the entire maxilla behind the bulla ossea nasolachrymalis, and opens into the sulcus semilunaris above a (now broken) processus uncinatus. The sphenoidal sinus occupies the whole of the presphenoid and extends into the alisphenoid and pterygoid processes. A moderate sized frontal air-cell extends forwards to gain the frontal bone, and opens below into the sulcus semilunaris anterior to the ostium maxillare. Two posterior ethmoidal cells, one small, one large, constitute the bulk of the ethmoidal lateral mass.

A second young gorilla cranium (R.C.S. 34.31) with milk dentition *in situ* and about the same size and age as specimen 30 A, shows an incipient frontal

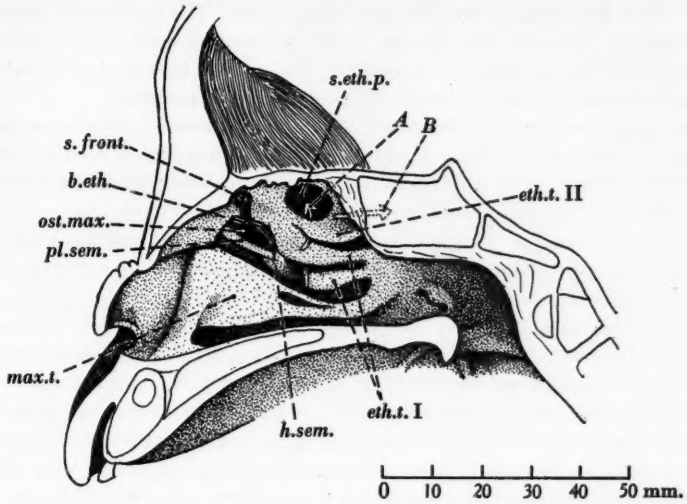


Fig. 15. Lateral nasal fossa wall of young gorilla (L.G.B.), wet specimen, with ethmoturbinal I partly removed, and frontal and posterior ethmoidal sinuses opened. Arrow A enters posterior ethmoidal sinus; B enters the sphenoidal sinus.

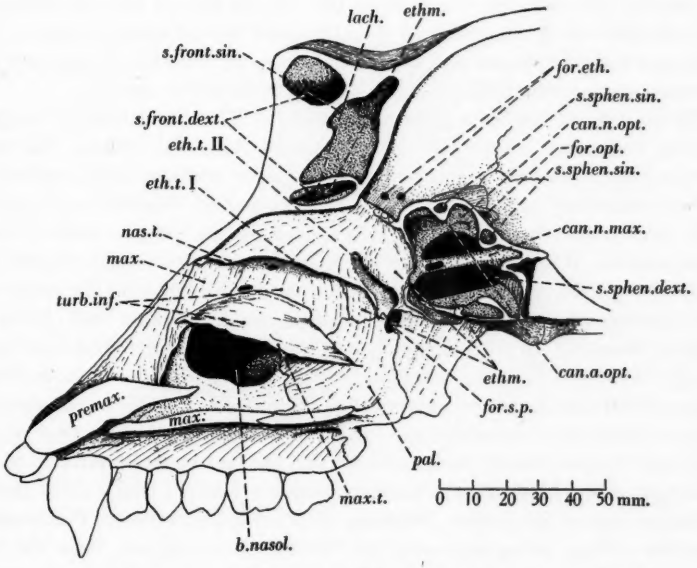


Fig. 16. Lateral nasal fossa wall of adult gorilla (St Thos. 1), macerated specimen, undissected.

sinus which has already reached the frontal bone. The sphenoidal sinus not only occupies the anterior moiety of the sphenoidal body but invades the ethmoid also; the maxillary sinus occupies the maxilla behind the dilated bulla nasolachrymalis. A large posterior ethmoidal cell is present.

An adult female gorilla cranium (R.C.S. 26.5) likewise presents capacious frontal and sphenoidal sinuses, and a maxillary sinus which occupies by extension the whole of the ethmoidal lateral mass. A single posterior ethmoidal cell, reduced by antral encroachment, occurs on the right side only.

In an adult male gorilla (R.C.S. 25) the frontal sinus is enormous and complicated; the sphenoidal sinus extends into the alisphenoid and pterygoid processes; the maxillary sinus invades the zygomatic bone laterally and the ethmoidal lateral mass superiorly, becoming thus contiguous with the frontal and sphenoidal cavities. On the right the true sphenoidal sinus is exceptionally small and the ipsilateral maxillary sinus has invaded the sphenoid by way of functional replacement. A single posterior ethmoidal cell is present bilaterally. A mature gorilla skull (C 1), of uncertain sex, presents a multilocular and somewhat complicated frontal sinus, a sphenoidal sinus extending into the alisphenoid and pterygoid processes, and a maxillary sinus which involves the greater part of the ethmoidal lateral mass. A single large posterior ethmoidal sinus opens into the superior meatus. In a huge mature male gorilla (B.M. unregistered) the frontal and sphenoidal sinuses are maximally developed. The antrum, which opens into the sulcus semilunaris above a well-developed uncinate process, fills not only the body of the maxilla behind the nasolachrymal bulla, but also the greater part of the ethmoidal lateral mass, becoming thus contiguous with the frontal and sphenoidal sinuses. A relatively large and still discrete posterior ethmoidal sinus opens into the superior meatus.

The macerated skull of a subadult animal (St. Thos. 1) exhibits a complete dentition and a full complement of unobliterated cranial sutures. The nasolachrymal duct presents a short uninflated upper portion continuous at the lachrymo-maxillary articulation with an enormously dilated lower portion (bulla ossea nasolachrymalis): the lateral wall of this bulla is formed chiefly by the maxilla, the medial wall by the inferior turbinate's vertical plate plus a small part of the palatine, whilst the bulla is closed above by the lachrymal. In the lateral bullar wall runs the infraorbital canal, the bony wall of which is partially deficient. In the middle meatus the processus uncinatus is attached at both its extremities, viz. anteriorly to the attachment of ethmoturbinal I and posteriorly to the lateral mass of the ethmoid. Thus in the recent state this specimen must have resembled specimen T.C.D. in possessing the type of plica semilunaris which extends backwards beyond the hiatus semilunaris, in contradistinction to the type of plica (seen in specimen L.G.A.) which stops short of the hinder end of the hiatus. Between its attached extremities the processus uncinatus is free, being separated by the hiatus semilunaris from the bulla ethmoidalis above, and by a fontanelle (closed in life by soft tissue) from the extensive vertical plate of the inferior turbinate below.

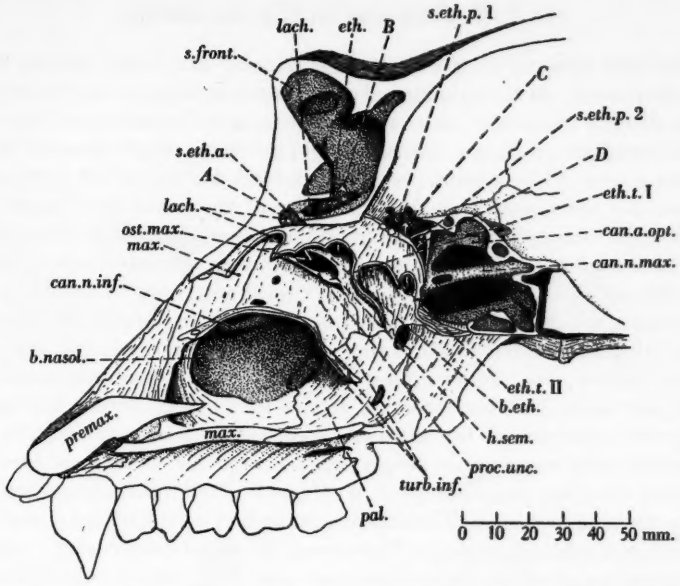


Fig. 17. Same specimen as preceding with maxillo-turbinal and ethmoturbinals I and II removed, and with frontal and ethmoidal sinuses opened. Arrow A enters anterior ethmoidal sinus; B enters frontal sinus.

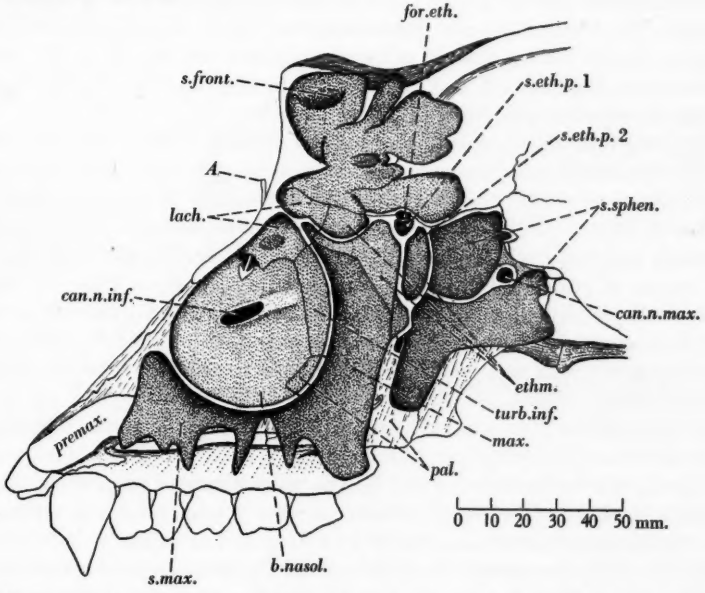


Fig. 18. Same specimen as preceding, dissected to plane of medial wall of orbit, showing sinuses and nasolachrymal bulla. Arrow A passes through the undilated portion of nasolachrymal duct to bulla nasolachrymalis.

The bulla ethmoidalis is a short ridge situated above and parallel to the uncinate process. At the anterior end of the hiatus semilunaris lies the entrance to the anterior ethmoidal sinus, an excavation into the lachrymal bone. The ostium maxillare presents a distinct margin inferiorly (where bounded by the superior margin of the vertical plate of the inferior turbinate) but elsewhere its margins are not demarcated from the floor of the sulcus semilunaris. The frontal sinus, occupying chiefly the frontal, but circumscribed in some part by both lachrymal and ethmoid, opens below between ethmoturbinal I and the bulla ethmoidalis. The maxillary sinus, occupied anteriorly by the bulla nasolachrymalis, is extremely large, occupying not only the maxilla but portions of the ethmoid and the palatine also. Each of the two posterior ethmoidal sinuses present reaches the orbital wall, and each is contiguous with the sphenoidal sinus. This last structure attains its maximal anthropoid ape development in the gorilla. In the present specimen it occupies entirely the large sphenoidal body and extends deeply into both the alisphenoid and pterygoid processes, effecting pneumatization of all parts of the sphenoid save only the orbito-sphenoid element, and establishing contiguity with the frontal, maxillary and posterior ethmoidal sinuses. The sinus is traversed by the bony canal containing the maxillary nerve, and the upper part of its lateral wall is depressed by the structures (optic nerve and ophthalmic artery) grooving the superficial aspect of the sphenoidal body.

Examination of other gorilla skulls reveals an identical type of nasal fossa structure. The uncinate process shows much variability, some skulls presenting the short, freely ending type of process responsible for the short plica semilunaris noted in specimen L.G.A., and others the long, doubly attached type of process, described in specimen St. Thos. 1 (see Fig. 19).

The suture between ethmoid and inferior turbinate may, and often does, become obliterated at a relatively early post-natal age—indeed its persistence in an animal so mature as the St. Thos. 1 specimen is unusual. That the intranasal sutures of this particular specimen have however been correctly identified, and that such sutures suffer but little change of position during the later stages of post-natal growth, is amply confirmed by a study of infant gorilla skulls, in particular by one (R.C.S. 34.4) wherein the deciduous incisors alone have erupted and the deciduous canines and first molars are but in process of eruption. (In this infant gorilla, the bulla nasolachrymalis is already characteristically distinct.)

A wet specimen of an adult (11 years old) female lowland gorilla ("Moina"), whose skeleton still retains traces of immaturity, presents nasoturbinal, maxilloturbinal and ethmoturbinals I and II. The inferior meatus receives the dilated nasolachrymal duct. The middle meatus presents a long, large and prominent plica semilunaris, bounded superiorly by a deep and co-extensive sulcus semilunaris, in the hinder moiety of which appears the narrowly oval ostium of the maxillary sinus. The plica semilunaris terminates postero-inferiorly by fusing with the ethmoidal portion of the lateral nasal fossa wall. Above the sulcus

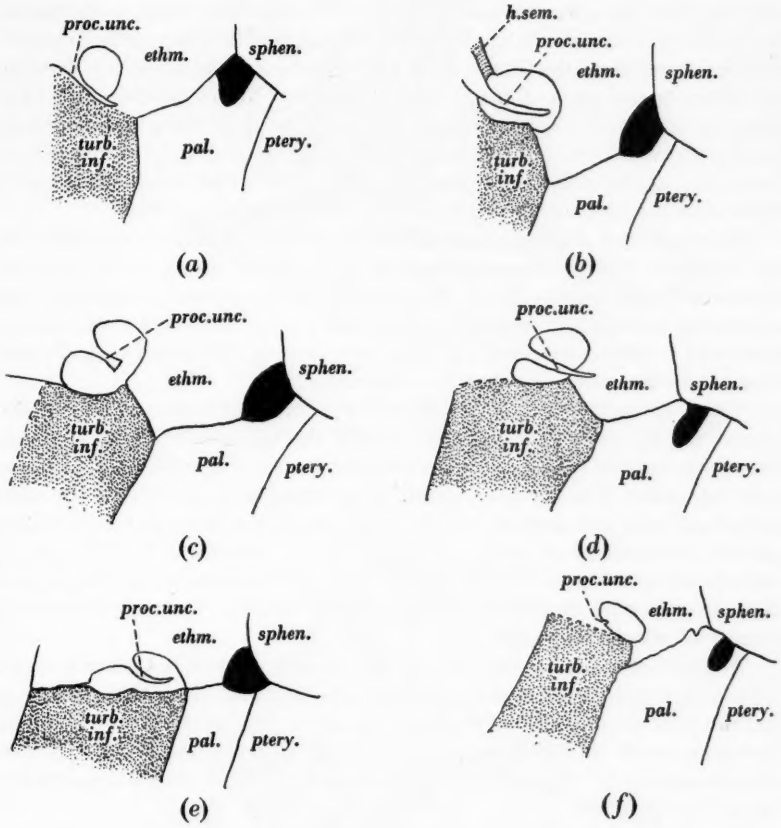


Fig. 19. Scheme showing variations of uncinate process in the gorilla: (*a*) adult male (B.M. unreg.); (*b*) adult (private coll.); (*c*) adult (St Thos. 1); (*d*) young (R.C.S. 30 A); (*e*) subadult (B.M. 3 K); (*f*) subadult female (R.C.S. 27).

semilunaris, between it and the ethmoidal bulla, the fronto-nasal duct opens into the middle meatus close to the attachment of ethmoturbinal I. The sphenoidal sinus, reduced by reason of the encroachment thereupon of the antrum, is divided by a median septum; each moiety opens by a small circular aperture into the spheno-ethmoidal recess. The maxillary sinus is enormous: not confined to the maxilla it invades the frontal, the ethmoid and the sphenoid. It is separated from the frontal sinus by a thin bony lamina only: it occupies the entire lateral mass of the ethmoid from nasal to orbital extremities and thence extends backwards to occupy almost the whole of the sphenoidal body, being separated from the reduced sphenoidal sinus proper by a thin curved bony lamina. No individual ethmoidal cells are anywhere recognizable, nor is there even suggestive evidence of their former presence.

The absence of distinct ethmoidal cells noted in "Moina" is paralleled by the condition of parts obtaining in certain macerated gorilla crania. Thus an adolescent female gorilla (R.C.S. 27) exhibits extensive frontal, maxillary and sphenoidal sinuses: the maxillary sinus invades the entire ethmoidal lateral mass and is contiguous with the other two sinuses. No traces of individual ethmoidal cells, past or present, are discernible.

A heavily crested adult gorilla skull (R.C.S. 22.2) presents a complex multilocular frontal sinus which invades almost the whole orbital roof: a large, irregularly septate sphenoidal sinus exhibits the usual alisphenoid and pterygoid extensions. The maxillary sinus, lying lateral and posterior to the nasolachrymal bulla and opening into the middle meatus above a distinct uncinate process, occupies by extension the whole of the ethmoidal lateral mass; it extends along the entire medial orbital wall, and is separated by thin osseous laminae only from the frontal and sphenoidal sinuses. No trace whatever of ethmoidal cells is detectable.

Another adult male gorilla (R.C.S. 20) presents essentially similar features. Frontal, sphenoidal and maxillary sinuses are characteristically developed: the antrum extends into the whole lateral mass of the ethmoid, thus becoming contiguous with the frontal sinus antero-superiorly and the sphenoidal sinus postero-superiorly. No ethmoidal cells occur nor is there even a suggestion of their former presence.

Broadly speaking, in the gorilla ethmoidal cells (which never exceed three in number in young skulls) may disappear, possibly with advancing age, and their place may be taken by a progressively enlarging maxillary antrum, whereas in man on the contrary, these cells maintain a persistent, life-long elaboration and extension. The very constancy of the gorilline frontal sinus (itself essentially an ethmoidal derivative) presupposes the general occurrence of at least one anterior ethmoidal cell, and all young specimens, wet or dry, examined by us present clearly established ethmoidal cells.

It is however equally possible that, in some gorilla specimens at least, middle and posterior ethmoidal cells do not appear at any stage of development, and that in such cases the antrum early attains its maximal extra-

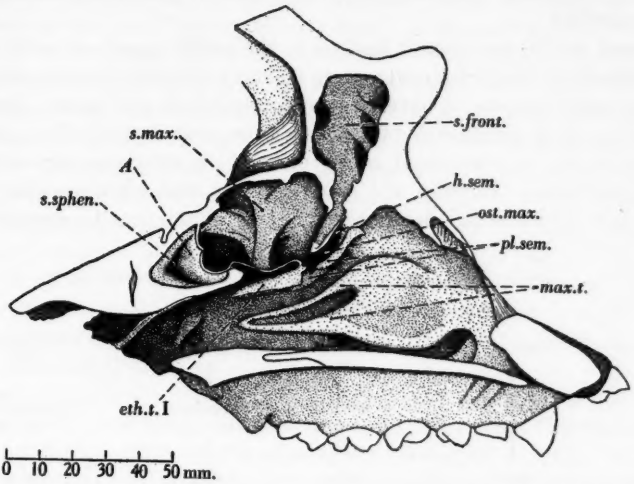


Fig. 20. Lateral nasal fossa wall of eleven years old female gorilla ("Moina"), wet specimen, as exposed by paramedian section. A = septum separating maxillary and sphenoidal sinuses.

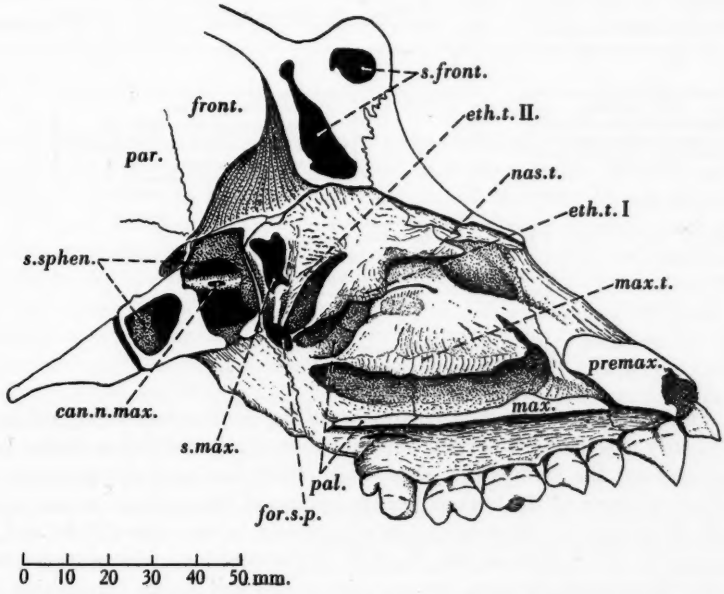
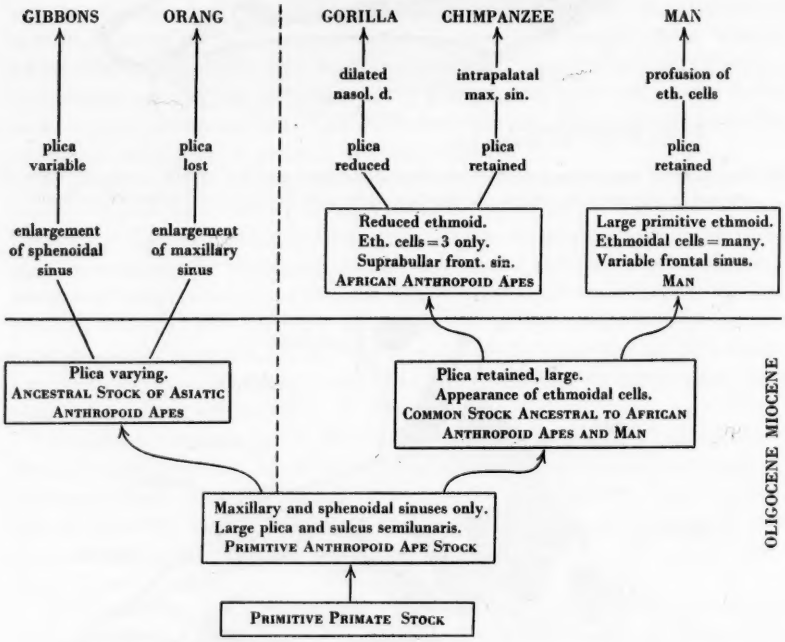


Fig. 21. Lateral nasal fossa wall of subadult female gorilla (R.C.S. 27), macerated specimen.

maxillary extension. Bauermeister (1939) failed to find ethmoidal cells in his gorilla material.

In most details our present findings in the gorilla agree well with those of earlier observers. Seydel's (1891) young animal is the only other specimen with an intact nasal mucosa to have been so far described and figured: this beast possessed a plica semilunaris of the long type, and Seydel called particular attention to the very hominoid arrangement of the structures surrounding its ostium maxillare. (Actually, the chimpanzee is even more man-like in this regard, but Seydel lacked chimpanzee material at the time he wrote.)

Table I. *Tentative phylogenetic scheme based on present findings*


GIBBONS
ORANG
GORILLA
CHIMPANZEE
MAN
dilated nasol. d.
intrapalatal max. sin.
profusion of eth. cells
plica variable
plica lost
plica reduced
plica retained
plica retained
enlargement of sphenoidal sinus
enlargement of maxillary sinus
Reduced ethmoid. Eth. cells = 3 only. Suprabullar front. sin. African Anthropoid Apes
Large primitive ethmoid. Ethmoidal cells = many. Variable frontal sinus. Man
Plica varying. Ancestral Stock of Asiatic Anthropoid Apes
Plica retained, large. Appearance of ethmoidal cells. Common Stock Ancestral to African Anthropoid Apes and Man
Maxillary and sphenoidal sinuses only. Large plica and sulcus semilunaris. Primitive Anthropoid Ape Stock
Primitive Primate Stock
OLIGOCENE MIOCENE

Keith (1902) gave the first illustration of the gorilline nasolachrymal bulla, previously described by Seydel, but unfortunately stated that a similar bulla characterized the chimpanzee also. Bolk (1917) has carefully described the successive stages of the bullous transformation of the gorilla's nasolachrymal duct, indicating, in old animals, its attainment to the orbital floor and the consequent direct opening into it of the lachrymal sac. Observations made during the present enquiry, however, do not support Bolk's supposition, but rather that of Aichel (1934) who correlates variations in size of the bulla with

racial rather than with age differences. Certainly, in quite young specimens, the bulla is already established, and may attain the floor of the orbit. The only authors to study in any detail the constitution of the osseous walls of both nasolachrymal duct and paranasal sinuses in the gorilla are Wegner (1936) and Wood Jones (1938); corresponding studies in other anthropoid apes appear to have been made for the first time during the present investigation. Unhappily, both these authors interpret as an intrinsic portion of the ethmoid that part of the inferior turbinate which forms the posterior wall of the nasolachrymal bulla, Wegner indeed specifically identifying it as the processus uncinatus. Now from a study of the specimens described above (particularly St. Thos. 1 and R.C.S. 34.4) such an interpretation appears plainly erroneous, for there can be no doubt as to the real nature of the bone in question. It is emphatically not the uncinate process, which is herein correctly identified for the first time since Seydel's (1891) paper.

If that portion of the inferior turbinate which participates in the formation of the wall of the nasolachrymal bulla were truly part of the ethmoid, then the ostium maxillare would indeed be entirely surrounded by ethmoid. It is upon such an hypothesis that Wood Jones (1938) has suggested (*a*) that the gorilline maxillary sinus is really an enlarged ethmoidal sinus, (*b*) that in this genus a true maxillary sinus is not developed. His conception of an ostium maxillare surrounded by ethmoid in the gorilla, but by maxilla in man, is untenable, for actually the ostium maxillare of the gorilla—like that of all other anthropoid apes and of man—lies between the ethmoid superiorly and the inferior turbinate inferiorly.

SINUSES OF MAN

Under this heading are discussed those points only which are relevant to an intelligible comparison of the paranasal sinuses in man and anthropoid apes. The human condition of affairs is most closely matched by the African anthropoid apes, and by the chimpanzee more particularly, since the gorilla lacks the well-developed plica and sulcus semilunaris of man and in addition manifests a peculiarly distinctive dilatation of its nasolachrymal duct—the well-known bulla ossea nasolachrymalis. The principal (often sole) opening of the human maxillary sinus into the sulcus semilunaris—the only opening to appear in the human embryo (Schaeffer, 1910*a*)—corresponds exactly to the anthropoid ape ostium maxillare; the human antrum, however, not infrequently displays one or more accessory ostia situated in the soft tissues which close the fontanelles in the lateral nasal fossa wall behind and below the uncinate process. Certain other deficiencies of the walls of neighbouring sinuses (e.g. maxillary and frontal) which occur occasionally in man are unknown in anthropoid apes.

Ethmoidal sinuses are much more profuse and elaborate in man than in the African anthropoid apes, wherein indeed their number probably never exceeds three. The human ethmoidal sinuses develop not only from the lateral walls of both the middle and the superior meatus, but frequently from the supreme

meatus also (Paulli, 1900; Schaeffer, 1910 *b*). The human frontal sinus shows considerable variability in its precise site of outgrowth from, and its communication with, the nasal fossa. It arises sometimes from a frontal recess of the middle meatus, or from a groove within that recess, sometimes from the anterior terminal recess of the sulcus semilunaris, and occasionally at least from a groove situated above the ethmoidal bulla but below the middle concha (Grünwald, 1912, types I, II and III). In every gorilla and chimpanzee specimen examined during this investigation the frontal sinus was found to arise as a diverticulum from this supra-bullar groove, and although present anthropoid ape material is too scanty to permit of certain pronouncement on the matter, it appears at least highly probable that such a suprabullar type of frontal sinus (the rarest form encountered in man) occurs most frequently, if not constantly, in the African anthropoid apes.

The understanding of the morphology of the human paranasal sinuses has been illuminated by detailed studies of their ontogenetic history (Killian, 1896; Mihalkovics, 1898; Kallius, 1905; Schaeffer, 1910 *a*, *b*; van Gilse, 1927). Soon after the appearance of the nasal conchae as eminences upon the ectodermal wall of the primitive nasal capsule a sulcus and a plica semilunaris are found (50–60 days stage), modelled at first in epithelium and soft tissues only and lacking any skeletal basis. Later (about the 65–70 days stage) the maxillary sinus appears as a localized outpouching of the wall of the sulcus semilunaris. In the mesenchymal tissues surrounding both sulcus and sinus cartilage is then deposited, so that the sulcus comes to lie between the processus uncinatus medially and the cartilaginous nasal fossa wall laterally, whilst the sinus comes to occupy the angle between this wall and the inturned margin thereof forming the cartilaginous framework of the inferior concha. (Such relationships are of course of a secondary nature, as ontogenetically the appearance of the cartilage is entirely subsequent to the primary modelling of the non-skeletal tissues.) Still later (4th month stage) the mucosa above the sulcus semilunaris bulges medialwards as the bulla ethmoidalis, and cartilage is developed beneath it. Meantime the maxilla itself (ossified in the 2nd month) remains separated from the developing maxillary sinus by the dense mesenchymal stratum wherein the cartilage of the lateral nasal wall afterwards develops; it is only by the subsequent resorption of this cartilage and the continued expansion of the sinus that the latter attains its first direct contact with the maxilla. With the ossification of the cartilaginous lateral nasal wall (in the 6th month) the maxillary sinus comes to lie between the ethmoid above and the inferior turbinate below, a secondary relationship dependent upon the mode of ossification of the pre-existent cartilaginous anlagen. (The remaining paranasal sinuses also make their initial appearance prior to ossification of the nasal fossa walls, but their history requires no discussion here.)

The early appearance of the sulcus semilunaris in the human embryo—paralleled by a corresponding precocity in other mammalian embryos (Peter, 1902)—suggests its primitive nature, and supports the thesis that both its

absence in the orang and certain gibbons, and its reduction in the gorilla, are in the nature of secondary degenerative modifications.

A review of nasal fossa embryology emphasizes the importance of the correct identification of mucosal folds, elevations, depressions and the like, as these early structures are surer criteria of sinus homologies than are the sutures between the tardily-appearing osseous elements of the lateral nasal wall.

Moreover, Paulli (1900) has shown that the maxillary sinus may establish contact with but a small part of the maxilla (as in *Hyrax* (*Procavia*), *Equus*, *Rhinoceros*, *Coelogenys*) or may fail entirely to establish any relationship with the maxilla (as in *Dicotyles*, *Hippopotamus*, *Cynaelurus* (*Acinonyx*), *Hystrix*), though in all these genera, the opening of the sinus, the sole reliable guide to its identity, occupies an essentially identical morphological position.

PHYLOGENETIC CONCLUSIONS

Certain conclusions concerning human and anthropoid ape phylogeny, derived from a study of the evidence presented in this paper, are indicated in Table I (*q.v.*). Admittedly such a table, based solely upon considerations of nasal sinus anatomy, must possess but limited intrinsic value: nevertheless, once constructed, a table of even this restricted nature is at least available for detailed comparison with others based upon wholly different lines of anatomical reasoning, and is therefore perhaps not unjustified.

An interpretation of paranasal sinus morphology alone yields strongly suggestive evidence that the primitive anthropoid ape stock gave rise to three divergent lines, leading respectively to the relatively primitive gibbons, to the highly specialized orang, and to the African anthropoid apes and man. Of the last group, the chimpanzee remains a relatively unspecialized member, the gorilla is distinguished particularly by an inflated nasolachrymal duct, whilst man is characterized by a remarkably complex ethmoidal labyrinth. The evidence of paranasal sinus structure, considered alone, is inadequate for the demonstration of the mutual relationships of these three forms, yet it does suggest the derivation of both chimpanzee and gorilla from an anthropoid ape common stock distinct from that leading to man. On the other hand, the common possession of ethmoidal sinuses suggests a close affinity between man and the African anthropoid apes to the exclusion of all other Primates.

The only comparable table based upon the evidence of nasal anatomy is that of Zuckerkandl (1887), who, erroneously believing ethmoidal sinuses to occur in *Mycetes*, suggested the derivation of the anthropoid apes from a common ancestor already equipped with such structures, and related possibly to *Anaptomorphus*. Zuckerkandl recognized the aberrant nasal structure of the orang and the specializations peculiar to man, but, from want of African material, could not, in his table, separate gorilla, chimpanzee and gibbon.

Selenka (1899), working on the skull as a whole, gave carefully compiled lists of both the primitive and the specialized characters exhibited by each

anthropoid ape. He recognized clearly the relatively primitive nasal structure of the gibbon and (apart from its innovation of frontal sinus development) of the chimpanzee: he suggested the possibility of a common origin of the chimpanzee and man from a dryopithecine ancestor.

The conclusions suggested in our table agree closely with those of most students of anthropoid ape structure, in particular those of Morton (1927), whose views were based upon comprehensive studies of the anthropoid ape foot, arm-body ratio, posture, dentition, skull and endocranial cast. His proto-anthropoid ape stage of the Oligocene corresponds to our "primitive anthropoid ape stock", and his dryopithecine stage of the Miocene to our "common stock ancestral to the African anthropoid apes and man".

Similar conclusions, emphasizing the close relationship between man and the African anthropoid apes through a Miocene dryopithecine stock, are set forth in Abel's table (1934), compiled on a strictly palaeontological basis.

On the other hand, our conclusions differ from those of certain other anatomists who have made particular studies of paranasal sinus anatomy. Thus both Weinert (1926) and Wegner (1936), mistakenly believing that orangs possess ethmoidal sinuses, failed to recognize these structures as a specialized neomorphic development linking man with the gorilla and chimpanzee. Wood Jones (1929) has denied any close relationship between man and the African anthropoid apes, and has argued against their derivation from any recent common ancestral stock. He stresses, for instance, the specialized cynomorphic reduction of the ethmoid in the anterior cranial fossa of the gorilla and chimpanzee, so marked a contrast to the human retention of a primitively large ethmoid. His later conclusion (1938) that the gorilline maxillary sinus is not homologous with that of man would, if correct, tend to separate these two forms, but reasons are given above for the rejection of this conclusion as untenable.

It is not denied that in their elaboration of ethmoidal sinuses and other structural details man and the African anthropoid apes may have pursued parallel evolutionary trends from a remote period. It is equally likely, however, if not indeed more probable, that the possession of such anatomical features indicates a real relationship through the medium of a common ancestral stock. The points emphasized by Wood Jones (1929) do indeed proclaim the close structural affinity between chimpanzee and gorilla, but the evidence of nasal anatomy compels the association of man with these forms.

On the functional side we have nothing to add to the suggestion made by Weidenreich (1924) and others that the paranasal sinuses are "dead" spaces in the skull, situated where but weak mechanical forces traverse the bones, and, for the sake of lightness, filled with air rather than with marrow.

SUMMARY

1. Wet and dry preparations from forty-eight juvenile and adult anthropoid ape specimens, representing all extant forms, have been examined.

2. All anthropoid apes tend to preserve the primitive primate heritage of paranasal sinus structure, viz. maxillary and sphenoidal sinuses only. The maxillary sinus opens always between the ethmoidal lateral mass superiorly and the inferior turbinate inferiorly: its ostium is primitively situated in a semilunar groove (sulcus semilunaris) guarded by a mucosal ridge (plica semilunaris) overlying the processus uncinatus, and thus opens indirectly into the middle meatus by means of this groove.

3. Some gibbons retain the primitive primate structural arrangement, others exhibit a reduction of the plica semilunaris so that the maxillary sinus opens directly into the middle meatus.

4. The orang skull is characterized by an enormous extension of the maxillary sinus which reduces or obliterates the sphenoidal sinus, and by a complete loss of the plica semilunaris.

5. The African anthropoid apes and man have acquired neomorphic ethmoidal sinuses, including a frontal sinus.

6. The chimpanzee presents a distinctive intrapalatal extension of the maxillary sinus; otherwise it preserves a type of paranasal sinus structure from which that of the gorilla and man may well have been derived.

7. The gorilla presents a dilated nasolachrymal duct, which encroaches upon the maxillary sinus, together with great variability in the degree of development of the plica semilunaris. The ostium of the maxillary sinus is disposed in typical anthropoid ape fashion.

8. Man is characterized by an extreme development and variability of the ethmoidal sinuses.

To Profs. A. B. Appleton, J. K. Jamieson and W. E. Le Gros Clark, we accord grateful acknowledgement of their generous loan of preserved gorilla material.

MATERIAL EXAMINED

		Text reference
Gibbons, wet		
(i) *Hylobates* sp.	Roy. Coll. Surg. Mus., Phys. Ser. E 165	R.C.S. E 165
(ii) *H. lar leuciscus*, ad. ♂	Roy. Coll. Surg. Mus., Phys. Store 30 A	R.C.S. 30 A
(iii) *H. lar leuciscus*, ad.	Roy. Coll. Surg. Mus., Phys. Store 30 B	R.C.S. 30 B
(iv) *H. lar lar*, juv.	Roy. Coll. Surg. Mus., Phys. Store 30	R.C.S. 30
Gibbons, macerated		
(i) *Hylobates hoolock*, ad. ♀	Roy. Coll. Surg. Mus., uncatalogued	D 1
(ii) *Hylobates hoolock*, ad. ♂	Private coll.	D 2
(iii) *Symphalangus syndactylus*, juv.	Roy. Coll. Surg. Mus., Comp. Ost. 60	R.C.S. 60

Orang, wet			
(i)	juv., incomp. milk dent.	Roy. Coll. Surg. Mus., Phys. Store 990	R.C.S. 990
(ii)	juv., perm. can. erupted	Roy. Coll. Surg. Mus., Phys. Ser. E 166	R.C.S. E 166
(iii)	juv., milk dentition	Roy. Coll. Surg. Mus., Phys. Ser. E 167	R.C.S. E 167
(iv)	ad. ♂	Roy. Coll. Surg. Mus., Phys. Ser. E 167*a*	R.C.S. E 167*a*
Orang, macerated			
(i)	juv., milk dentition	Roy. Coll. Surg. Mus., Comp. Ost. 54	R.C.S. 54
(ii)	ad. ♀	Roy. Coll. Surg. Mus., Comp. Ost. 44.6	R.C.S. 44.6
(iii)	ad. ♂	Roy. Coll. Surg. Mus., Comp. Ost. 45	R.C.S. 45
(iv)	ad. ♂	Roy. Coll. Surg. Mus., Comp. Ost. 39	R.C.S. 39
(v)	subad. ♀	Roy. Coll. Surg. Mus., Comp. Ost. 40 B	R.C.S. 40 B
(vi)	ad. ♂	St Thomas's Hospital, Anat. Mus.	St Thos. 3
Chimpanzee, wet			
(i)	ad.	Roy. Coll. Surg. Mus., Phys. Store 898	R.C.S. 898
(ii)	juv.	Roy. Coll. Surg. Mus., Phys. Ser. E 168 (1st perm. molars *in situ*)	R.C.S. E 168
(iii)	subad. ♀	Roy. Coll. Surg. Mus., Phys. Ser. E 168.2	R.C.S. E 168.2
Chimpanzee, macerated			
(i)	juv., milk dentition + 1st perm. molars	Private coll.	C 2
(ii)	juv., milk dentition	Private coll.	C 3
(iii)	immat. ♀	Private coll.	C 4
(iv)	ad. ♂, old	Private coll.	C 5
(v)	immat., ♀	Roy. Coll. Surg. Mus., Comp. Ost. 10.4	R.C.S. 10.4
(vi)	immat.	Roy. Coll. Surg. Mus., Comp. Ost. 12.55	R.C.S. 12.55
(vii)	ad. ♀	Roy. Coll. Surg. Mus., Comp. Ost. 6	R.C.S. 6
(viii)	ad. ♂	Roy. Coll. Surg. Mus., Comp. Ost. 8	R.C.S. 8
(ix)	ad. ♂	Roy. Coll. Surg. Mus., Comp. Ost. 9	R.C.S. 9
(x)	ad. ♂	Roy. Coll. Surg. Mus., Comp. Ost. 10.5	R.C.S. 10.5
(xi)	ad. (? sex)	Roy. Coll. Surg. Mus., Comp. Ost. 12.53	R.C.S. 12.53
Gorilla, wet			
(i)	Infant, milk dentition		L.G.B.
(ii)	juv., milk dentition + 1st perm. molars, Trinity College, Dublin		T.C.D.
(iii)	juv. (*c.* 4 years) first perm. molars fully erupted		L.G.A.
(iv)	immat.	Roy. Coll. Surg. Mus., Phys. Ser. E 168.5	R.C.S. 168.5
(v)	ad., ♀, 11 years	Roy. Coll. Surg. Mus., uncatalogued	"Moina"
Gorilla, macerated			
(i)	baby, milk incisors only	Roy. Coll. Surg. Mus., Comp. Ost. 34.4	R.C.S. 34.4
(ii)	juv., milk dentition	Roy. Coll. Surg. Mus., Comp. Ost. 30 A	R.C.S. 30 A
(iii)	juv., milk dentition	Roy. Coll. Surg. Mus., Comp. Ost. 30.31	R.C.S. 30.31
(iv)	immat., ♀, Gaboon	Roy. Coll. Surg. Mus., Comp. Ost. 27	R.C.S. 27
(v)	ad., ♀	Roy. Coll. Surg. Mus., Comp. Ost. 26.5	R.C.S. 26.5
(vi)	ad., ♂	Roy. Coll. Surg. Mus., Comp. Ost. 25	R.C.S. 25
(vii)	ad., ♂	Roy. Coll. Surg. Mus., Comp. Ost. 20	R.C.S. 20
(viii)	ad., ♂, Kivu	Roy. Coll. Surg. Mus., Comp. Ost. 22.2	R.C.S. 22.2
(ix)	ad. (? sex)	Private coll.	C 1
(x)	ad., ♂	St Thomas's Hospital Anat. Mus.	St Thos. 1
(xi)	immat.	Brit. Mus. (N.H.) Ost. Room, 3 K	B.M. 3 K
(xii)	ad. ♂	Brit. Mus. (N.H.) Mammal Gallery, uncat.	B.M. unreg.

KEY TO FIGURES

b.eth.	bulla ethmoidalis	*nas.t.*	nasoturbinal
b.nasol.	bulla nasolachrymalis	*ost.max.*	ostium of maxillary sinus
can.a.opt.	canal for ophthalmic artery	*pal.*	palatine
can.n.inf.	canal for infraorbital nerve	*par.*	parietal
can.n.max.	canal for maxillary nerve	*pl.sem.*	plica semilunaris
can.n.opt.	canal for optic nerve	*premax.*	premaxilla
dext.	right	*proc.unc.*	uncinate process
ethm.	ethmoid	*ptery.*	pterygoid
em.car.	carotid eminence	*sin.*	left
eth.t. I	ethmoturbinal I	*s.eth.a.*	anterior ethmoidal sinus (cell)
eth.t. II	ethmoturbinal II	*s.eth.p.*	posterior ethmoidal sinus
eth.t. III	ethmoturbinal III	*s.eth.p.* 1	posterior ethmoidal sinus 1
for.eth.	ethmoidal foramen	*s.eth.p.* 2	posterior ethmoidal sinus 2
for.opt.	optic foramen	*s.front.*	frontal sinus
for.s.p.	sphenopalatine foramen	*s.max.*	maxillary sinus (antrum)
front.	frontal	*s.sphen.*	sphenoidal sinus
h.sem.	hiatus semilunaris	*sphen.*	sphenoid
lach.	lachrymal	*temp.*	temporal
max.	maxilla	*turb.inf.*	inferior turbinate
max.t.	maxillo-turbinal	*zyg.*	zygomatic

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A NOTE ON THE LENGTH OF THE BASILAR MEMBRANE IN MAN AND IN VARIOUS MAMMALS

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In all authoritative textbooks, whether dealing with anatomy, physiology or otology, there is usually provided some information on the *width* of the basilar membrane at various levels of the ductus cochlearis or scala media. But, with very few exceptions, the *length* of the basilar membrane is not given or even mentioned, for the clear reason that direct measurements are difficult, if not impossible. Bethe (1926) states that the length of the basilar membrane in the human cochlea is 33·5 mm. without any indication of the method of measuring the length but with a reference to Retzius. This latter author (1884) gives the length of the whole ductus cochlearis as "about 36 mm.", again without explaining how the figure was arrived at. Most writers on the subject have accepted Retzius's figure as authoritative and his original statement is perhaps the only source from which any information as to the length of the basilar membrane has ever been derived. The mammalian cochleae which are described by Retzius with a great wealth of histological detail are those of man, rabbit, ox, pig and cat, but only in connexion with the human cochlea does he mention the length of the ductus cochlearis. Later writers have justifiably taken the length of the ductus cochlearis as being the same as the length of the basilar membrane.

The ductus cochlearis winds in a spiral manner around the truncated cone represented by the modiolus (Fig. 1). Sections of the whole cochlea enable one to obtain the radius of the base of the cone, the perpendicular height of the cone and the number of coils. It seems that there should be no special difficulty in obtaining a mathematical formula for calculating the length of a spiral wound round the cone. But when it comes to actual application it will be found that the formulae for such spirals are extremely complicated. There are two kinds of "regular spirals". In one case the same distance is preserved between adjacent coils, so that the spiral moves upwards more and more steeply. In the second case the spiral moves up at an unchanged angle so that the coils come closer together.

There are two reasons why it is hardly possible to use such formulae for determining the length of the basilar membrane: (1) the number of turns are comparatively few and it is not possible to decide whether the cochlea belongs to the first or to the second type of spiral; (2) an examination of a series of mammalian cochleae shows quite clearly that the spirals are not always "regular" ones, i.e. if the margins of the successive coils are joined on the microphotographs, a straight line is not always obtained. A simpler method was therefore devised which will become clear after examining Figs. 1 and 2.

Fig. 1 represents a diagrammatic "3-turn" cochlea with a millimetre scale which would make its size not unlike that of many mammals. In Fig. 2 the ductus cochlearis is drawn as a spiral line in one plane with the distances *a–b*, *c–d* and *e–f* the same as in Fig. 1. The ductus cochlearis is seen six times in one plane of section. The spiral line shows that the ductus cochlearis must make at least $2\frac{1}{2}$ complete turns if, for example, it begins at point *a* and ends at point *f*. On the other hand, it might make almost $3\frac{1}{2}$ turns without any further portion of it appearing in the plane of section represented by the line *a–c–e–f–d–b*. Therefore if the ductus cochlearis appears six times in a section through the modiolus, we speak of it as having three turns, i.e. midway between the minimum and the possible maximum. The "3-turn" cochlea is seen in cat and dog, a "$2\frac{1}{2}$-turn" cochlea in man, calf, sheep, and a "4-turn" cochlea in the guinea-pig.

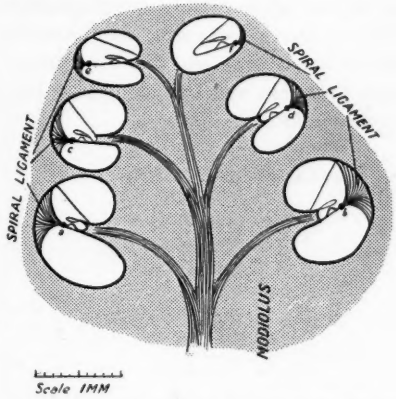

Fig. 1. Diagrammatic section of a cochlea with three turns.

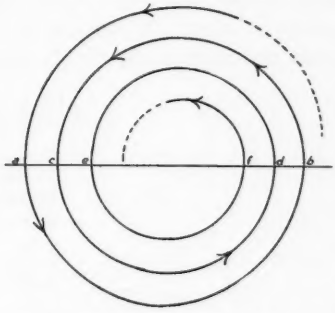

Fig. 2. The ductus cochlearis of the cochlea in Fig. 1 shown as a diminishing spiral line in one plane.

The simple and approximate method of determining the length of a spiral around a cone is to look upon each turn as a complete circle and to base calculations on the diameters of the succeeding "circles". In order to obtain the diameter of each "circle" as regards the cochlea, there must be agreement on the points in the ductus cochlearis from which the measurements are to be made. When examining sections of cochleae, a region that is easily identified is the point of attachment of the basilar membrane to the spiral ligament; in other words the outer margin of the basilar membrane. This point stands out even if the preparation is not a specially good one as regards the organ of Corti. The inner end of the basilar membrane cannot be so easily decided, for it is often difficult to say where the bony spiral lamina ends and where the basilar membrane begins. It is suggested, therefore, that the diameter for each turn of the cochlea shall be considered to be the distance between the outer edge of the basilar membrane on one side to the corresponding point on the opposite side.

These are the distances *a–b*, *c–d* and *e–f* in Fig. 1. An additional advantage of agreeing on this point in each ductus cochlearis is that one actually measures the length of a line along the edge of the basilar membrane. The circumference of each "circle" is obtained by multiplying the diameter by the constant 3·14. The circumferences of the successive turns are added together. If the last turn is not complete, half the circumference of the previous turn is added. In this way the total length of the basilar membrane is arrived at. An example of such a calculation may be given, using Fig. 1 as a basis. Distance *a–b* measured by the scale of 1 mm. is 3·2 mm., *c–d* is 2·6 mm. and *e–f* is 1·8 mm. The circumferences of the successive three turns are 10, 8·1 and 5·6 mm., giving the total length of the basilar membrane as 23·7 mm., say 24 mm.

It is obvious that only sections which show the whole of the modiolus and which pass through all the turns will be suitable for determining the length of the basilar membrane. Microphotographs are all taken at the same magnification. The points selected for measuring the successive diameters, viz. the outer edges of the basilar membrane, are perfectly easily seen and direct measurements are made. In the case of the human cochlea and the cochlea of five other mammalian species, the results were as follows:

Length of basilar membrane

	mm.
Human	32
Guinea-pig	21
Sheep	35
Cat	20
Dog	24
Calf	33

When allowance is made for the divergence in size of these mammals there is a remarkable similarity of the basilar membrane measurements in this short series. The cochleae as a whole resemble each other to an extraordinary degree, irrespective of body-size, size of head or weight of brain in each species. The same applies to measurements of the width of the basilar membrane, as I showed in a previous communication.

More than 60 years ago Retzius gave the length of the ductus cochlearis as being "about 36 mm." He did not state what method he used for obtaining this measurement; it may have been by employing a complicated mathematical formula, or in a more simple manner such as is suggested here. It may conceivably have been some form of direct anatomical measurement after defining the bony cochlea by filing down the hard petrous bone around it, or Retzius may have dealt with the decalcified petrous bone. Whatever procedure was employed, the figure 32 mm., which I have obtained for the length of the basilar membrane in the human cochlea, shows that the simple method put forward here is adequate for ordinary purposes. Further, the figures for the five other mammalian species may also be accepted as reasonably accurate.

The sections of cochleae from which the microphotographs were taken are at the Ferens Institute of Oto-Laryngology, Middlesex Hospital, London. I have to thank Mr F. J. Cleminson, the Director of the Institute, for his kind permission to use them.

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THALAMIC DEGENERATION INDUCED BY TEMPORAL LESIONS IN THE CAT

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The connexion between the medial geniculate body and the cortex of the temporal lobe of the brain was demonstrated by Monakow (1895) and has been studied in the primates and rodents by several investigators. Woollard & Harpman (1939) made lesions in the medial geniculate bodies of two cats and used the Marchi method to trace degenerated fibres to the sylvian and middle ectosylvian and the dorsal part and anterior ectosylvian gyri. The boundaries of the auditory area thus determined are approximately the same as those of the area from which potential changes induced by sound stimuli may be recorded with the oscillograph (Körnmuller, 1937; Bremer & Dow, 1939). Degeneration of cells in the medial geniculate body has been observed in the cat following a lesion of the dorsal parts of the sylvian gyri (Mettler, 1932). Further information concerning the disposition of chromatolysis resulting from lesions in and near the acoustic cortex may be useful in future functional studies.

METHOD

The medial geniculate bodies in six cats were examined for cell reaction following eight lesions of the temporal cortex, two of the animals having lesions on both sides. In each case a period of about two weeks was allowed to elapse after the operation before the animal was killed. Three brains were embedded in paraffin, sectioned at 25 μ and mounted on slides in sets of every tenth section, and the other three were embedded in nitrocellulose, sectioned at 50 μ and mounted in sets of every eighth section. All the sections were stained with thionin. The medial geniculate degeneration in each case was marked on a set of form drawings of the thalamic nuclei as seen in sections 1 mm. apart in the frontal plane of the Horsley-Clarke stereotaxic instrument (Figs. 1–4).[1]

THE NORMAL MEDIAL GENICULATE BODY

The auditory nucleus in the thalamus of the cat is an oval structure about 4 mm. long in a rostro-caudal direction. It lies between frontal planes 3 and 7 of the Horsley-Clarke instrument (3 and 7 mm. rostral to a line connecting the centre of the external auditory meatuses), and consists of two distinct parts. Its rostral and caudal ends and the lateral part of its middle section consist of

[1] The assistance of Mr A. J. Scherman in preparing the sections, and of Mr W. W. Meyers in making the line drawings, is greatly appreciated.

small cells (*gs*). The ventromedial part in planes 4–6 (*gl*) is composed of large cells and is apparently homologous with the parageniculate nucleus which has been described in the monkey (Clark, 1936; Rundles & Papez, 1938). The rostral half of the large-cell nucleus is adjacent to a prominent indentation, or hilus, occupied by the fibres of the auditory radiation (*AR*) and the brachium of the inferior colliculus.

The rostral end of the medial geniculate body is blended with the caudal end of the ventrolateral nucleus of the thalamus (*vl*, Fig. 2, A 7.5). The dorsomedial boundary, where the medial geniculate body adjoins the pars posterior of the lateral nucleus (*lp*), is also difficult to locate. No outline is given in the drawings for a suprageniculate nucleus, the territory of which (as described by Rioch, 1929) is included in the ventral part of *lp* (Fig. 2, A 5.5). Some of the dorsomedial corner of the medial geniculate body (*gl*, A 5.5, *gs*, A 4.5) also may properly belong to the suprageniculate nucleus (see Ingram *et al.* 1932) since no complete degeneration was found in this region.

RESULTS

Nearly complete degeneration of the medial geniculate body following the large lesion on the left side in cat 124 demonstrated that both the large and the small cells send fibres to the cortex. Extensive geniculate chromatolysis resulting from lesion 124 L in the middle ectosylvian gyrus and lesion 125 R in the dorsal parts of the sylvian gyri indicated the position of the terminals of the bulk of the auditory radiation fibres (Fig. 5).

Dorsally the auditory area is bounded by the middle suprasylvian sulcus; lesions dorsal to the sulcus in the middle suprasylvian gyrus (124 L, 125 R, 127 R and L) caused cell reaction in the lateral posterior nucleus and pulvinar and, if the lateral gyrus was involved, in the dorsal nucleus of the lateral geniculate body (Waller & Barris, 1937; Waller, 1940). Chromatolysis in the caudal part of the lateral geniculate body in 124 R (not illustrated) was probably caused by interruption of the optic radiation fibres as they pass beneath the temporal cortex. The lesion extended deep into the white matter, and it is known that deep temporal injuries involve the optic fibres in the human brain. The same explanation may be applied to the extensive lateral geniculate degeneration in 124 L, in which only a small piece of the lateral gyrus was removed.

That the acoustic area extends rostrally into the dorsal part of the anterior ectosylvian gyrus was shown by degeneration of cells in the rostral part of the medial geniculate body after the lesion in cat 123. Cats 125 and 126 had lesions of the lower part of the anterior ectosylvian gyrus on the left side (not illustrated), but no effect was observed in the auditory nucleus. The results from these and other lesions of the rostral part of the brain are described in a separate communication (Waller, 1940). The lower boundary of the auditory area is near the dorsal tip of the sylvian sulcus, since lesions 121 and 127 R

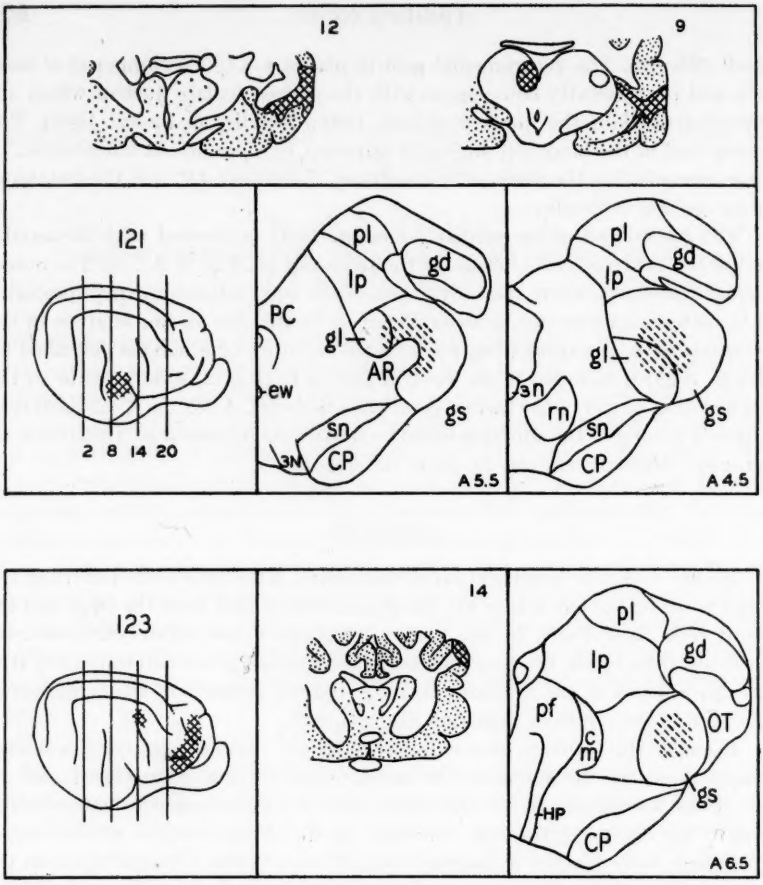

Fig. 1. Cat 121. Lesion of the posterior sylvian gyrus on the right side, and the resulting partial degeneration of the central portion of the medial geniculate body. The effects of the lesion of the left medial thalamic nucleus are not considered in this paper.

Cat 123. Small lesion of the upper end of the right anterior ectosylvian gyrus, and the resulting partial degeneration in the rostral end of the right medial geniculate body. The effects of the frontal lesion, and of one cortical and one midbrain lesion on the left side, are not considered in this paper.

In Figs. 1–4 the lesions are indicated by cross-hatching, partial degeneration by diagonal interrupted lines, and complete or nearly complete degeneration by diagonal continuous lines. The right side is to the right in the drawings of sections. Each section is accompanied by a number giving its distance in millimetres rostral to the interaural line, or zero frontal plane of the Horsley-Clarke stereotaxic instrument. Small numbers accompanying the drawings of the lateral surfaces of brains 121 and 127 give the rostral distances of the vertical lines. Abbreviations: *ar*, arcuate nucleus; *AR*, auditory radiation; *cm*, centrum medianum; *CP*, cerebral peduncle; *ew*, nucleus of Edinger and Westphal; *gd*, lateral geniculate body; *gl*, large cell nucleus of the medial geniculate body; *gs*, small cell part of the medial geniculate body; *HP*, habenulo-peduncular tract; *ip*, interpeduncular nucleus; *lp*, lateral nucleus pars posterior; *OT*, optic tract; *PC*, posterior commissure; *pf*, parafascicular nucleus; *pl*, pulvinar; *rn*, red nucleus; *sn*, substantia nigra; *sub*, subthalamic nucleus of Luys; *vl*, ventrolateral nucleus; 3*n*, oculomotor nucleus; 3*N*, oculomotor nerve; 3*V*, third ventricle.

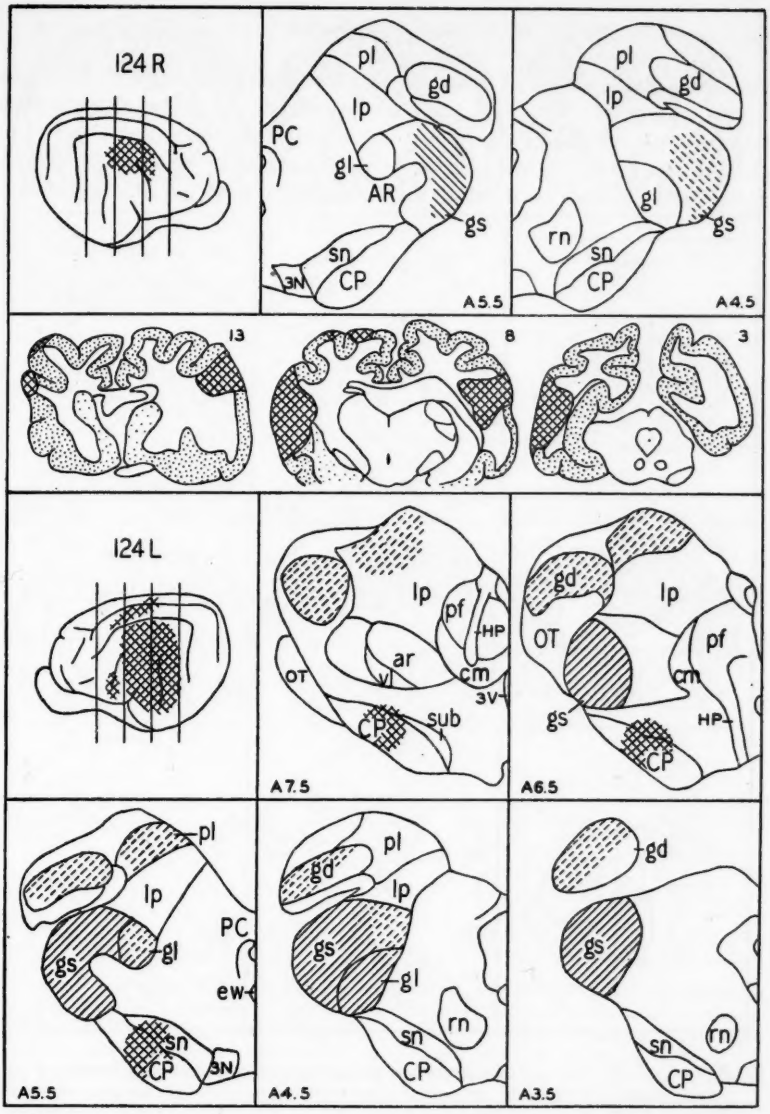

Fig. 2. Cat 124. Above are shown the middle ectosylvian lesion and the area of chromatolysis in the lateral part of the medial geniculate body on the right side. The region of cell reaction extended into the rostral end of the geniculate nucleus, which is not shown. There was partial degeneration of the caudal end of the right lateral geniculate body, in sections caudal to A 4.5. Below are shown the large temporal and small suprasylvian and lateral gyrus lesions on the left side, and the resulting degeneration of most of the cells of the medial geniculate body and some of those of the lateral posterior nucleus, pulvinar and lateral geniculate body. The effects of the electrolytic lesion of the cerebral peduncle are not considered in this paper.

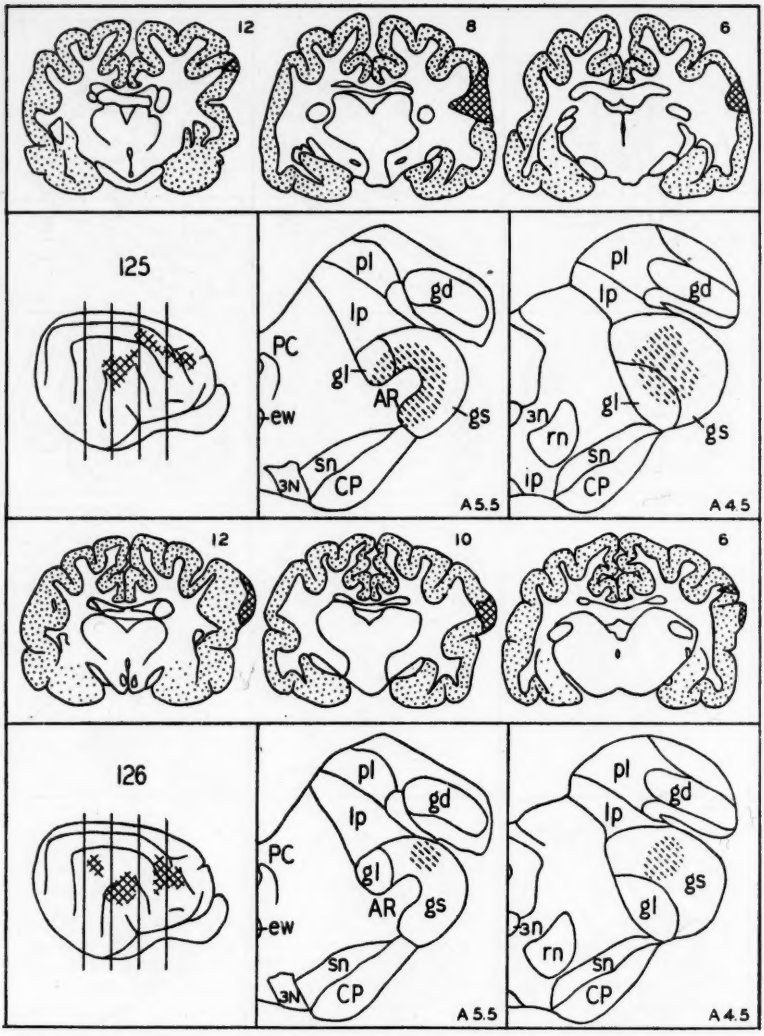

Fig. 3. Cat. 125. The region of degeneration in the right medial geniculate body resulting from the sylvian lesion extended into the central part of the rostral end of the nucleus, which is not illustrated. Chromatolysis in the rostral part of the lateral posterior nucleus (A 8–10) caused by the suprasylvian injury is not shown. Besides two frontal injuries not considered in this paper, this animal had also a left anterior ectosylvian lesion (see text).

Cat 126. The lesion of the upper part of the posterior ectosylvian gyrus was the one intended; the sylvian lesion was made accidentally, and was quite shallow. The mild cell reaction in the medial geniculate body was probably a result of the sylvian lesion, since it was located in the region which degenerated in cat 125. Besides the right anterior suprasylvian lesion marked in the drawing, this animal had also a left anterior ectosylvian lesion (see text) and a left frontal cortex injury.

caused some geniculate degeneration, while 127 L, which was placed more ventrally and behind the sulcus, had no effect.

A small posterior ectosylvian lesion (126 R) was made for the purpose of locating the caudal boundary of the acoustic area, but the results were disappointing because the sylvian gyri were accidentally injured. The sylvian

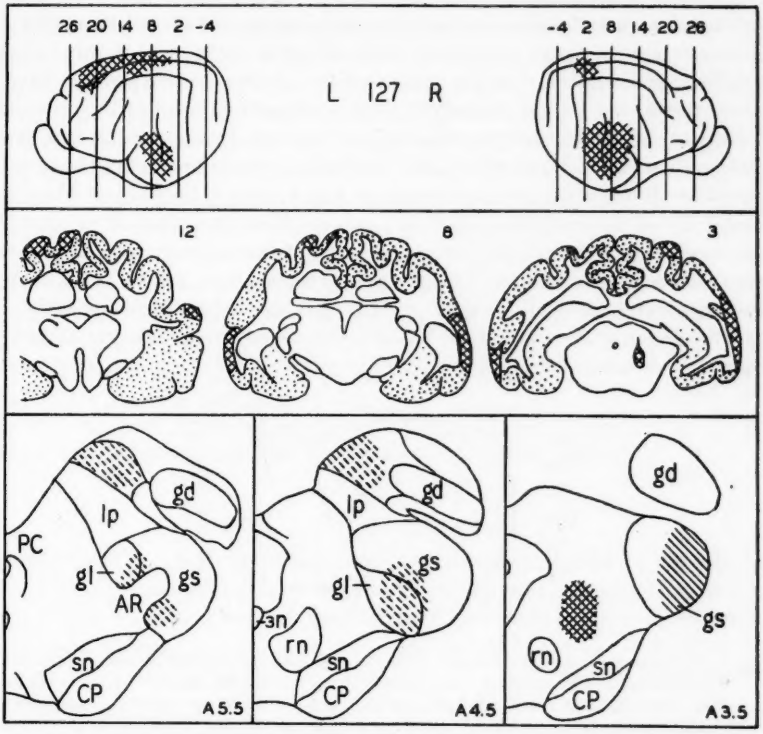

Fig. 4. Cat 127. Lesions were made in the right suprasylvian gyrus and lower temporal region, and caused mild chromatolysis in the pulvinar and the central part of the medial geniculate body. At A 3.5 the lateral part of the geniculate body was completely degenerated, while the extreme caudal tip, and also sections about A 4, showed only mild chromatolysis. Since it is barely possible that the tegmental lesion at A 3.5 could have had something to do with this result, it is included in the drawing for the sake of completeness. When the brain was removed at autopsy large areas of cortical atrophy were found on the left side, as shown in the drawing of the left side of the brain. There was no reaction in the left medial geniculate body; the lateral geniculate and lateral posterior nucleus degeneration are described in another paper.

injury was not as large as its area on the surface drawing of the brain (Fig. 3) seems to indicate, since much of it was very shallow. The resulting geniculate degeneration was probably caused by the accidental rather than by the intended lesion, since its position is similar to that of the degeneration caused by

the sylvian lesion in 125 R. If this deduction is correct there was no effect from the posterior ectosylvian lesion, and the auditory area does not extend posterior to the upper end of the posterior ectosylvian sulcus.

Some degree of localization within the auditory radiation is apparent from the results of these experiments. Lesion 123, in the rostral part of the acoustic area, caused chromatolysis in the rostral end of the geniculate body. The caudal end of the auditory nucleus was not affected by lesions 124 R, 125 R and 126 R, which covered the upper parts of the sylvian and ectosylvian gyri, but some of its cells degenerated on the right side in cat 127. In one section near the caudal end of the medial geniculate body (A 3.5) in this animal most of the cells were chromatolytic, while sections 0·5 mm. rostral and caudal to this one were practically normal. Since it is hardly likely that this reaction could have been caused by the nearby tegmental lesion (see Fig. 4), it may be concluded that the caudal tip of the geniculate nucleus sends its fibres to cortex in or near the posterior ectosylvian sulcus. The central part of the auditory nucleus and the large cells degenerated after ventrally placed lesions (121, 125 R, 127 R), while the large middle ectosylvian lesion (124 R) did not disturb the large cells.

The effects of the electrolytic lesions of the brain stem (121, 124 L, 127 R) are to be considered in another paper.

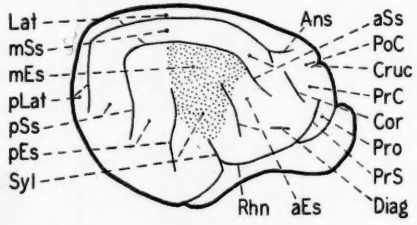

Fig. 5. The extent of the cat's acoustic cortex as demonstrated by the thalamic degeneration method is marked by stippling. *Ans*, ansate sulcus; *Cor*, coronal sulcus; *Cruc*, crucial sulcus; *Diag*, diagonal sulcus; *aEs*, *mEs*, *pEs*, anterior and posterior ectosylvian gyri and sulci and middle ectosylvian gyrus; *Lat*, lateral gyrus and sulcus; *pLat*, posterolateral gyrus and sulcus; *PoC*, *PrC*, postcrucial and precrucial gyri; *Pro*, gyrus proreus; *PrS*, presylvian sulcus; *Rhn*, rhinal fissure; *Syl*, sylvian gyri and sulcus; *aSs*, *mSs*, *pSs*, anterior, middle and posterior suprasylvian gyri and sulci.

DISCUSSION

The acoustic area of the cat's cortex as outlined by the thalamic degeneration method (Fig. 5) coincides with the area in which Bremer & Dow (1939) found potential changes generated by sound stimuli. It is smaller than the region from which Davis (1934) and Gerard *et al.* (1936) recorded auditory impulses. Its anterior, dorsal and posterior boundaries are dependably established through the agreement of results obtained with the Marchi method (Woollard & Harpman, 1939) and the oscillographic method (Körnmuller, 1937) and the cytological studies of Körnmuller (1937) and Bremer & Dow

(1939). As to the lower boundary, the thalamic degeneration method gave results favouring the conclusion that the hearing area reaches to the dorsal end of the sylvian sulcus, including a small bit of cortex which, according to Bremer & Dow, differs structurally from the main dorsal part of the area.

The cortical area which receives the terminals of the auditory fibres is relatively larger in the cat, as well as in rodents (Wallèr, 1934; Pennington, 1937; Stoffels, 1939) than it is in primates (Poliak, 1932; Clark, 1936; Walker, 1937; Walker & Fulton, 1938; Rundles & Papez, 1938). It is commonly agreed that there is some localization in the auditory radiation, various parts of the medial geniculate body having individual areas of connexion in the cortex. Point-to-point specificity is suggested by the work of Ades *et al.* (1939), who showed that different parts of the cat's medial geniculate body are concerned in transmission of impulses initiated by vibrations of different frequencies. In their experiments conduction of impulses from low-frequency stimulation was impaired by lesions placed ventrally and medially in the geniculate body. This observation indicates that low frequencies activate the large-cell nucleus, which forms the ventromedial part of the geniculate body and sends fibres to the lower part of the auditory cortex. With respect to the rostral end of the medial geniculate body, the localized connexion to the rostral part of the acoustic cortex is similar to that of the rat (Waller, 1934). The dorsolateral part of the auditory nucleus apparently sends its fibres to the dorsal part of the cortical area for audition (see Poliak, 1932).

SUMMARY

The location of the auditory area in the cerebral cortex of the cat was determined by comparing the positions of eight surgical lesions of the temporal cortex with the distribution of the resulting cellular degeneration in the medial geniculate bodies. Six cats were used, two having lesions on both sides. The auditory radiation from the thalamus ends in a cortical area including the middle ectosylvian gyrus and the dorsal parts of the sylvian and anterior ectosylvian gyri, extending ventrally to the upper tip of the sylvian sulcus. The rostral tip of the medial geniculate body is connected to the rostral part of the auditory area in the upper end of the anterior ectosylvian gyrus. The fibres from the central and ventromedial part of the geniculate body, including the large-cell portion of the nucleus, end in the lower part of the acoustic area in the sylvian gyri.

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THE DISTRIBUTION OF DISTURBANCES OF SWEAT SECRETION AFTER EXTIRPATION OF CERTAIN SYMPATHETIC CERVICAL GANGLIA IN MAN[1]

By L. GUTTMANN

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EXACT knowledge of the topographical relations of the sympathetic nervous system and the sweat glands in man is still scanty. Earlier workers made no special and systematic study of the subject, and it is only since surgeons became interested in the problems of the sympathetic nervous system that any attempt has been made to obtain detailed knowledge. Braeucker (1928), developing the classical researches of Langley (1891–1922), showed, after section of grey rami, that the grey rami of C. 7, C. 8, Th. 1, and L. 4, L. 5, S. 1, S. 2, are responsible for the sweat secretion of the hand and foot respectively. Adson & Brown (1929), Foerster (1929), Lewis & Landis (1930), Guttmann (1930), Wilson (1934), Lewis (1938) and List & Peet (1938 *a*) have reported the results on sweating of removing certain sympathetic ganglia and portions of the sympathetic chain, the resulting disturbances of sweating being studied by some of these workers by colorimetric methods. More cases must be carefully investigated, both before and after the operation, in order to obtain a better view of this field.

Below are reported the effects on sweat secretion which follow extirpation of certain cervical sympathetic ganglia with and without stripping of the common carotid artery. Particular attention will be given (1) to the topographical relations existing between the different parts of the cervical sympathetic chain and the secretory skin areas; and (2) to the disturbances of sweat secretion, not only in the denervated area but also in the other, intact parts of the sweat gland system.

In the earlier cases the method employed was Minor's iodine-starch method, but since 1933 I have used the more simple chinizarin method. The technique of this method has been described in previous papers (Guttmann, 1937). In the majority of the cases an examination of the sweat secretion was performed before and after operation. Preoperative examination is not always feasible, but its importance in the study of the autonomic system in man is shown by some of the cases in this paper.

[1] This work was undertaken with the aid of grants from Balliol College, Oxford, and from the Society for the Protection of Science and Learning.

ISOLATED EXTIRPATION OF THE SUPERIOR CERVICAL GANGLION

The significance of the topographical relations between the superior cervical ganglion and its corresponding skin areas as an indicator for surgical treatment is illustrated by the following case:

Case 1. A woman, aged 54 years, had suffered for several years from pains in the right side of the face, especially in the forehead. At the outset the pains were spasmodic, but later became almost continuous. They were worse during changes of weather. The patient herself had noticed that when the pains were intense there was increase of sweating in the painful area, especially in the forehead, and this was accompanied by a flow of tears from the corresponding eye.

The neurological examination revealed no pathological signs other than widening of the right palpebral fissure. Superficial and deep sensibility were intact. All reflexes were normal. X-ray photographs of the skull were normal. No pathological signs related to the internal organs were evident: blood pressure 140/80: Wassermann reaction negative.

1*a*

1*b*

1*c*

Fig. 1*a–c*.

The thermo-regulatory sweating dye-test (hot tea, aspirin and radiant-heat cradle) showed a distinct hyperhydrosis on the right side of the face and neck which is illustrated in Fig. 1*a*, a photograph taken at the beginning of the experiment. The hyperhydrosis was most marked in the forehead region.

Noting the especially clear localization of the pains in the forehead region, an operation was performed for the avulsion of the right supra-orbital nerve and its frontal branch. Fig. 1*b* shows the absence of sweating in the area of supply of this nerve 14 days after operation. In other respects the picture shows the same state as before the operation, namely great hyperhydrosis of the right side of the face and neck, while in the shoulder region the intensity of sweat secretion is the same on the two sides.

Four weeks after the avulsion, as there was no lessening of the pains, the right superior cervical ganglion was removed. After this operation there was a great improvement. In the course of the succeeding 5 years the patient only had slight disagreeable sensations when the weather changed, and these were not to be compared in severity with the earlier pains.

The sweating dye-test about 3 weeks after this operation showed a complete anhydrosis of most of the region previously hyperhydrotic in the experiment with hot tea, aspirin and radiant-heat cradle (Fig. 1*c*). The picture also shows that in this case there was now an increase of sweat secretion in the area of supply of the adjacent part of the sympathetic chain, namely the right middle cervical ganglion. This reaction will be considered later. The right Horner's syndrome also is clearly seen in Fig. 1*c*, contrasting with the widening of the palpebral fissure of Fig. 1*a* and 1*b*.

These findings show that isolated extirpation of the superior ganglion abolishes thermo-regulatory sweat secretion in an area which includes the first

Fig. 2.

three cervical dermatomes. A similar observation has been made by List & Peet (1938*a*). On the other hand, in a case studied by me, after my former colleague, Dr Altenburger, had removed the left superior cervical ganglion on account of otosclerosis and tinnitus, the anhydrosis was not complete. In the thermo-regulatory sweating test about 4 weeks after operation (Fig. 2), a small secretion of sweat still occurred in the region of the left forehead and upper lip. This is difficult to explain. It is possible that the extirpation was incomplete, or there may be anatomical variations such as occur in the lower part of the cervical trunk.

EXTIRPATION OF THE SUPERIOR AND MIDDLE CERVICAL GANGLIA WITH AND WITHOUT STRIPPING OF THE COMMON CAROTID ARTERY

In 1934 Wilson, using the lens method, demonstrated anhydrosis of the left side of the face and neck together with a considerable part of the left shoulder region after resection of the cervical sympathetic chain between the superior and middle ganglia. List & Peet (1938 *a*) described a case in which there was a stab wound in the supraclavicular fossa. In addition to a distinct weakening of certain upper-arm muscles there was anhydrosis of the left side of the face and the left shoulder. The authors suppose that the lesion involved

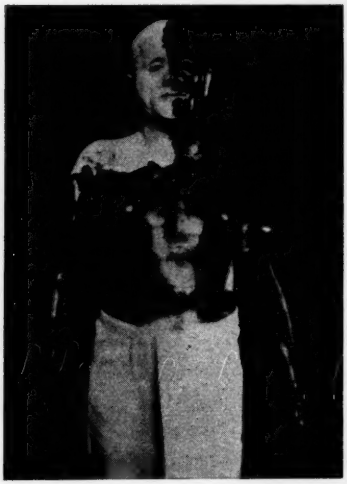


Fig. 3.

Fig. 4.

the sympathetic chain at the level of the middle cervical ganglion. Alternatively, they considered "that the ascending chain was severed above the middle cervical ganglion and the grey rami for the 4th and 5th cervical nerves were divided".

Clear experimental demonstration of the functional relation of the middle cervical ganglion to the sweat glands of its corresponding skin areas is given in the following cases:

Fig. 3 shows a case in which the superior and middle ganglia and their intermediate trunk had been removed on account of severe causalgia after amputation of the right hand caused by gunshot wound (Prof. Foerster). The removal of the inferior ganglion was not attempted. The common carotid artery was also stripped of its sympathetic plexus.

The sweating dye-test before the operation showed an increase of sweating of the right forearm, especially around the amputation stump. A fortnight after sympa-

thectomy (Fig. 3) the sweating dye-test revealed complete anhydrosis not only of the right side of the face and neck but also of the whole shoulder region as far as the superior part of the upper arm, together with the upper part of the chest down to the second rib. The same result was found 2 and again 5 months after sympathectomy. The interesting result of the pilocarpine experiment in this case will be considered later.

I found an analogous loss of thermo-regulatory sweating in a woman, aged 32 years (case 4). In this case the superior and middle ganglia with their intermediate trunk were removed on account of unilateral migraine which had begun after the patient's first confinement (Fig. 4). The carotid artery was not stripped. No symptoms of disorder of the nervous system or of the internal organs could be found before the operation. The thermo-regulatory sweating dye-test before the operation revealed no disturbances of sweat secretion. Three weeks, 6 months and 11 months after ganglionectomy, the disturbances of thermo-regulatory sweat secretion were identical with those of case 3. This clear result in both of the cases shows that the middle cervical ganglion has no connexion with the cervical 6th dermatome, as was formerly accepted in the literature. It supplied in these cases only the 4th and a great part of the 5th cervical dermatome. But it is possible that there are individual variations in the area of supply of the middle ganglion.

EXTIRPATION OF THE SUPERIOR, MIDDLE AND INFERIOR GANGLIA

The sweat disturbances resulting from the extirpation of the whole cervical sympathetic chain, including the stellate ganglion, are illustrated in the following case:

Case 5. In this case, that of a man aged 36 years, already mentioned in one of my former papers (1930), operation was carried out because of diffuse violent pains in the right arm and shoulder and the right side of the face, caused by a gunshot injury of the right shoulder-blade with resulting chronic suppuration of the bone (Prof. Foerster). There were no signs of a brachial plexus or spinal cord lesion. All conservative and local surgical treatments had so far been unsuccessful. In this case, besides the removal of the sympathetic chain, a large piece of the common carotid artery was stripped.

The sweating dye-test 2 and 6 weeks after the sympathectomy shows that, besides the face, shoulder and upper part of the chest, the whole upper limb was included in the area of anhydrosis. The sweat secretion was preserved only in the medial part of the upper arm (Fig. 5).

The same results were found in another case operated upon by Foerster (case 6). Here the operation had been performed on a woman, aged 38 years, because of severe diffuse pains in the right arm and shoulder and the right side of the face, resulting from a high degree of kyphoscoliosis with severe spondyloarthritis. No clinical symptoms of any brachial plexus or spinal cord lesion were to be found. After sympathectomy in this case, in contrast to the case shown in Fig. 5, the remaining sweat secretion on the medial side of the upper

arm did not extend so far distally but was limited to the lateral wall of the axilla. The reason for this difference between the two cases is to be found in the fact that in the latter the second thoracic ganglion had also been eliminated from its function, whilst in case 5 this ganglion was preserved. A similar case (case 7) is seen in Fig. 6.

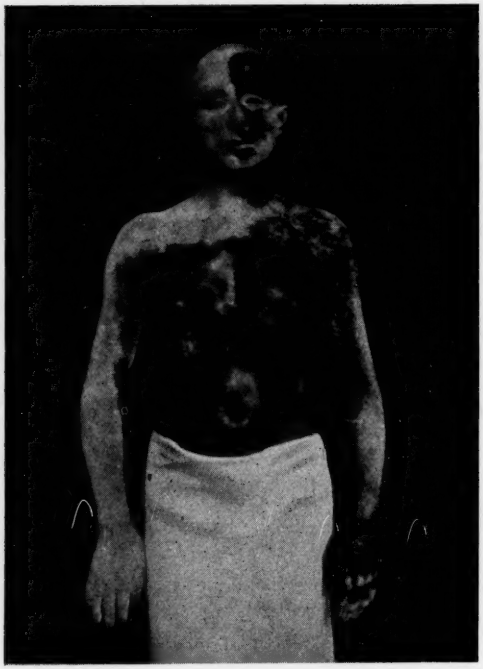


Fig. 5.

Fig. 6. Amputation after gunshot wound. Sympathectomy for causalgia (chinizarin method).

These examples show how accurately the sweating dye-test reveals the functional topographical relations between the single elements of the sympathetic chain and their corresponding secretory areas in the skin. This is not of merely theoretical interest. For the surgeon who requires to verify the extent of his operative measures on the sympathetic nervous system, this is the surest and most simple method. Those with some experience of this branch of surgery know that during an operation it may be very difficult to determine the extent of the resection of the sympathetic chain.

LESIONS OF THE SYMPATHETIC CHAIN DUE TO STRETCHING

The dye-test is of special value for the objective demonstration of certain lesions of the sympathetic nervous system caused by severe stretching of the spine and the extremities (Zerrschädigung). During and since the last war cases have been reported of lesions of peripheral nerves and of the spinal cord due to stretching (Auerbach, Foerster, Reinhard, Guttmann). My observations show that lesions of the sympathetic chain can also be caused by a stretching mechanism, and without obvious involvement of the adjacent peripheral nerves. The following two cases may help to draw attention to this type of lesion.

Case 8. A mechanic, aged 37 years, met with the following accident: While he was handling the flywheel of a machine his right arm was snatched by the wheel and was violently swung with it. The man did not faint, but after the accident he complained of pains in the right shoulder, extending into his right arm as well as into the right side of his neck and face.

The neurological examination about 3 weeks after the accident revealed a Horner's syndrome on the right side and a weakness of elevation and adduction of the right shoulder. In the resting position the right shoulder was a little lower than the left. The general power of the right arm, especially when being raised above the horizontal, was diminished. There was no disturbance of sensation and reflexes, or of the electrical response of muscles.

The sweating dye-test showed a distinct hypohydrosis of the right side of the face, neck and upper part of the chest and of the lateral parts of the right upper arm (Fig. 7). On the other hand the medial parts of the right upper arm and forearm and the back of the hand sweated a little more than on the left side. In this case it may be concluded that there was an incomplete lesion in the middle part of the cervical sympathetic chain above the stellate ganglion (Fig. 7).

Case 9. A farmer, aged 46 years, whose antecedents do not show any particular features, met with the following accident: He was walking along the high road beside his cart when his horses shied at a passing motor car and bolted. The man fell down and with his right hand hanging in the reins he was dragged along for a considerable distance, and his right arm was badly stretched. He immediately felt some weakness and pain in his right arm. Voluntary power returned soon after the accident, but the pains remained constant, and at times became violent and almost unbearable.

The neurological examination about 6 weeks after the accident did not show any pathological symptoms apart from a slight widening of the right palpebral fissure and slight diminution of the general power in his right arm. There was no atrophy of muscles, and no disturbance of sensation or of reflexes. The X-ray examination of the spine did not reveal any abnormality. The sweating dye-test, however, showed a considerable diminution of the thermo-regulatory sweat secretion on the right side of the face, shoulder, upper part of the chest and the whole arm and head, with the exception of the medial parts of the upper arm; in other words, there was an incomplete lesion of the cervical sympathetic chain at the level of the stellate ganglion (Fig. 8).

These cases provide clear evidence of damage to the cervical sympathetic chain due to violent traction on the upper limb, and without any gross lesion of the adjacent peripheral nerves. In lesions of this type accurate diagnosis is

impossible without recourse to the sweating test, or other methods of study of the sympathetic system. During the last war such cases as these, and analogous cases of nerve damage due to missiles sticking in the adjacent bone (Prellschädigung), often remained unrecognized, or were diagnosed as functional disorders for lack of adequate methods of examination.

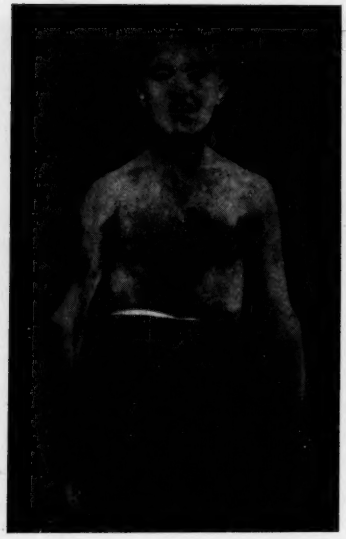


Fig. 7.

Fig. 8.

THE BORDER ZONE REACTION

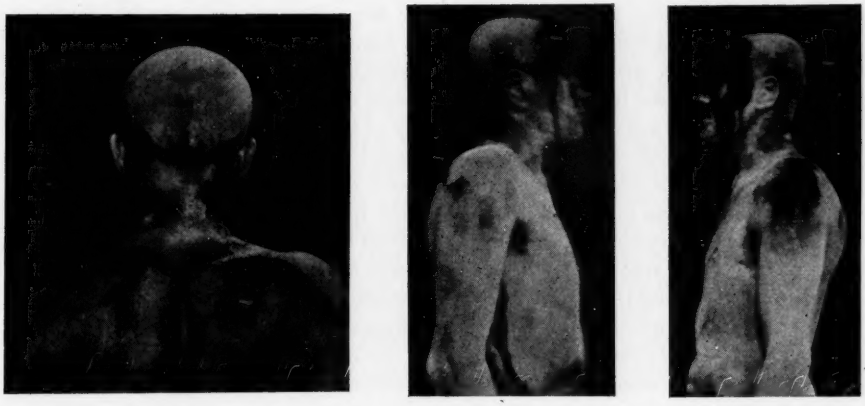
Extirpation of the sympathetic ganglia has interesting effects on the nervous mechanism of the regulation of perspiration balance. In addition to the loss of thermo-regulatory sweat secretion in the sympathectomized skin area there is also hyperactivity of those areas of sweat glands which are anatomically and functionally connected with the denervated anhydrotic area. This hyperactivity affects not only the zones adjacent to the area of anhydrosis on the ipsilateral side but also the areas on the contralateral side corresponding to the anhydrotic area.

During the first days after the extirpation of the ganglia the disturbance of the perspiration balance sometimes appears as a more or less considerable spontaneous sweating of these perilesionary border zones. This was especially noticeable in case 3 on the contralateral normal side of the face, and in the ipsilateral border zone of the anhydrosis on the chest. After a longer or shorter interval the spontaneous sweating disappears, but there remains an increased

irritability of these zones, which can be shown by producing sweating by any method. This can be demonstrated in the first stages of the sweating dye-test by a specially rapid onset and an increase of sweat secretion in these parts as compared with others.

After extirpation of the superior cervical ganglion the sweat glands of the ipsilateral shoulder, upper arm and upper part of the chest, and, in addition, those of the contralateral side of the face, come into a state of secretory hyperactivity (Fig. 1*c*).

After extirpation of the superior and middle cervical ganglia the perilesionary hyperhydrosis develops on the ipsilateral side in those parts of the upper arm which are adjacent to the area of anhydrosis as well as on the forearm and chest (Fig. 4). In case 3 (Fig. 3) the perilesionary hyperhydrosis



9*a* 9*b* 9*c*

Fig. 9*a–c*.

could not be demonstrated because at the end of the experiment the sweat of the various areas ran together. In this case, however, the perilesionary hyperactivity was clearly proved by means of the pilocarpine sweating dye-test which followed subcutaneous injection of 15 mg. pilocarpine (Fig. 9). In this experiment the reaction was obvious, particularly in those parts of the normal left shoulder and upper arm corresponding to the denervated parts of the right shoulder and upper arm. The sweat secretion induced by pilocarpine was noticeable on the denervated side, especially in the face, but it was diminished on this side especially in the right shoulder and the right upper arm. The sweating was more obvious apart from the right axilla in the corresponding regions of the normal left shoulder and upper arm. The pilocarpine experiment was carried out about 4 weeks after the ganglionectomy (Fig. 9*a–c*).

In all other cases of ganglionectomy described in this paper the pilocarpine dye-test, performed 2–4 weeks after the operation, showed also a

hypohydrosis of the denervated side following subcutaneous injection, although the sweat secretion (as in case 3) began first in this area. In these cases, after subcutaneous pilocarpine injection a higher room temperature caused a stronger general sweat secretion; on the other hand, the earlier onset of sweat secretion on the denervated side was not influenced by differences of room temperature. In the literature there is no general agreement about the effects of pilocarpine on a sympathectomized skin area and this question requires further investigation (see Langley, 1922; Burn, 1922; Kohler & Weth, 1924; Guttmann & List, 1928; Adson & Brown, 1929; Lewis & Landis, 1930; Wilson, 1936; List & Peet, 1938*b*).

After the extirpation of the stellate ganglion the perilesionary hyperhydrosis on the ipsilateral side extends to the chest and the trunk. On the contralateral side the arm and especially the hand, as well as the face, may show an increased sweat secretion (Fig. 6). In Fig. 5, however, the differences on the trunk are no longer distinct as the sweat of the various parts had run together.

The question arises whether the perilesionary secretory hyperactivity appears in an equal intensity in the ipsilateral and contralateral border zones, or whether there are differences in intensity of this reaction. Among the cases described three different conditions were found. (1) The reaction of the border zones occurred in an equal intensity on both sides of the body. (2) In some cases it showed an increased intensity in the ipsilateral border zone. (3) The reaction was more marked in the contralateral border zones of the anhydrotic area. A general rule cannot be enunciated from these results. Nevertheless, this question should be studied on a larger scale. In those cases in which a difference in the intensity of the secretory hyperactivity could be observed, the phenomenon was strongly marked not only by a specially strong sweating, but also by its rapid response within the respective border zones.

Another question concerns the distance to which the secretory hyperactivity extends in the ipsilateral parts adjacent to the area of anhydrosis. It varies in different cases. Fig. 6 shows how far the hyperactivity may extend. The ipsilateral side of the sympathectomized area shows not only a specially strong hyperhydrosis of the immediately adjacent dermatomes Th. 3 and Th. 4, but the hyperhydrosis extends (although to a lesser degree) as far as the 10th thoracic dermatome. By no means all the factors affecting the various reactions are known. It would seem that, apart from a constitutional disposition, variations in the underlying pathological lesion may play a decisive part.

COMMENT

In discussing the nature and mechanism of the secretory hyperactivity in the border zones, attention must first be drawn to the fact that the transformation of function of the sweat glands in the ipsilateral and contralateral border zones does not merely arise from the elimination of certain parts of the sympathetic chain. This phenomenon is always to be found in cases of peripheral nerve section, and in the area surrounding a scar, as well as in many lesions of

more central parts of the nervous system, e.g. with cases of radicular, spinal and cerebral lesions (Guttmann & List, 1928; Guttmann, 1933). These observations are in conformity with some observations by Gask & Ross (1934) and by Lewis (1938) concerning changes of sweat secretion in the upper limbs after unilateral and bilateral ganglionectomy, and with others concerning changes in the skin temperature and heat regulation after bilateral splanchnic sympathectomy (Roth *et al.* 1939). The last-mentioned authors actually found a complete reversal of the skin temperature of the fingers of the upper and lower extremities after extensive sympathectomy. They were able to show "that the regulatory control of the elimination of heat, in order that the temperature of the body may remain practically constant at 98·6° F., has been shifted from the lower to the upper extremities when the patient was placed in environmental conditions (65–85° F.) which demand body cooling and vasomotor regulation". The authors also noticed in their case an increased moisture of the hands after sympathectomy of the lower extremities. Hence the transformation of the secretory function of the border zones seems to be the expression of a regular and systematic kind of reaction of the organism and may be considered as analogous to other reactions in the vegetative sphere, e.g. to vasomotor regulation. As the sweat secretion in man is an essential factor in heat regulation, it may be assumed that the disturbance of the perspiration balance, resulting from the deficiency of a more or less considerable area of secretion, also causes a permanent disturbance of heat regulation. This, however, is prevented by a correcting function of the nervous system, whereby the sudomotor mechanism in close relation with the anhydrotic area comes into a state of compensatory hyperactivity. In this way the balance of the disturbed connexions of the sympathetic system is regained, and the ensuing adaptation of the whole sudomotor system guarantees now the maintenance of a normal heat regulation.

As to the development of the nervous process responsible for the border zone reaction, there are still two questions experimentally to be solved: (1) whether or not similar or even the same reflex mechanism is responsible for its formation as W. Hess (1931) and others have described in connexion with the blood flow; and (2) whether the hyperactivity of border zones is produced by means of a local axone reflex or spinal and supraspinal reflexes. Considering the secretory hyperactivity of border zones as part of an extensive co-ordinating phenomenon of adaptation and compensation, I do not believe that this phenomenon is adequately explained by assuming that merely a local axone reflex is responsible for its formation. For, apart from the fact that the reflex hydrosis occurs also in corresponding parts of the contralateral side, the extent and intensity of the secretory hyperactivity are generally not greater than the total demand of sweat secretion required in order to maintain the perspiration balance. This, however, requires the intercalation of some moderating nerve impulses, i.e. the activity of a reflex mechanism which acts both in a synergistic and in an antagonistic way. This kind of co-ordinated activity of the nervous

apparatus is, however, hardly conceivable by means of a mere local axone reflex, but must be induced by the mediation of spinal and supraspinal reflexes.

This interpretation of the nature of the border zone reaction is not incompatible with the fact that such a regulating activity of the nervous system can be prevented by exogenous as well as endogenous factors. This is especially to be noted in cases where there is some indication of a general neuropathic condition. In other words, the secretory hyperactivity of border zones loses its character as a regulating and compensating phenomenon as soon as the general innervatory balance is disturbed. In these circumstances the development of a general excessive and very unpleasant reflex hydrosis may result, even in cases in which the lesion affects only a very small part of the nervous system. I was able to observe this phenomenon in the case of a young gynaecologist, who, in 1930, fell on a fragment of glass and severed the volar superficial branch of his left ulnar nerve. He had suffered from tuberculosis, having experienced two slight haemoptyses in 1928–9. A hyperhydrosis of the border zones corresponding to the loss of the small secretory area within the denervated area of supply of the small cutaneous nerve was by no means the only effect. There even developed a considerable and most unpleasant reflex hydrosis over the whole body. At this time signs of active tuberculosis were no longer present. It is interesting to note that the general hyperhydrosis receded only gradually, with the regeneration of the small cutaneous nerve.

SUMMARY

Extirpation of certain cervical sympathetic ganglia causes disturbances of sweat secretion in clearly limited cutaneous areas; the latter can easily and accurately be revealed by the colorimetric examination of sweat secretion (iodine-starch method or chinizarin method).

Isolated removal of the superior cervical ganglion produces anhydrosis of the face and the upper part of the neck. The lower limit of anhydrosis corresponds to the 2nd or 3rd cervical dermatome.

With simultaneous extirpation of the middle cervical ganglion the anhydrosis extends over the neck and shoulder region as far as the proximal part of the upper arm, and reaches beyond the clavicle to the second rib. Hence the anhydrosis includes the whole of the 4th and part of the 5th dermatome. The question whether and how far the middle ganglion supplies also the 6th cervical dermatome requires still further observations.

If the extirpation includes the inferior cervical ganglion, the anhydrosis involves the whole of the upper limb except the medial part of the upper arm. Hence the area from the 5th cervical to the 1st thoracic dermatome is affected by the extirpation. The extent to which the medial part of the upper arm is involved in the disturbance of sweat secretion depends on how far the 2nd and especially the 3rd ganglion have been eliminated.

The anhydrosis involves only thermo-regulatory sweating. In all the cases mentioned here, subcutaneous injection of 15 mg. of pilocarpine 2–4 weeks after the sympathectomy produced distinct though reduced secretion on the sympathectomized side, but the sweat secretion on this side began earlier than on the normal side.

The extirpation of the sympathetic ganglia produces not only a loss of thermo-regulatory sweat secretion in a limited skin area; the elimination of a considerable part of the skin which is necessary for the reflex regulation of body temperature leads to a reaction of other parts of the sudomotor system as well.

The development of a secretory hyperactivity of the zones adjacent to the area of anhydrosis can be observed both on the ipsilateral and contralateral sides. This perilesionary secretory hyperactivity of the border zones is considered to be a part of an extensive and co-ordinated functional adaptation—a compensation phenomenon serving to maintain the perspiration balance and normal heat regulation. The localization and extent of the border zone reaction, as well as the reflex mechanism underlying it, have been discussed.

I wish to thank Prof. Hugh Cairns for providing opportunities for this work and for his kind assistance.

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Anatomical Society of Great Britain and Ireland

PROCEEDINGS

April 1940

RECORDED BY

A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

APRIL 1940

The Annual General Meeting of the Society for the Session 1939–40 was held at the Royal College of Surgeons, Lincoln's Inn Fields, London, at 3 p.m. on Friday, 26 April and at 10 a.m. on Saturday, 27 April 1940. The President (Professor J. P. Hill) in the Chair.

Dr P. Bacsich read a paper on "Some observations on the Bodian silver method with an account of a new modification". The modified procedure is as follows:

Part I. Preliminary treatment. The material is fixed for 24 hr. in a mixture of 5 c.c. acetic acid, 5 c.c. conc. formalin, 90 c.c. of 80 % spirit: it is then washed for 24 hr. in 70 % spirit, then taken through 95 % spirit and three changes of absolute alcohol (plus 2 % crystalline carbolic acid) and embedded in 2 in. celloidin (plus 2 % crystalline carbolic acid) for 24 hr. It is then washed for a few minutes in ether-alcohol and given three changes of chloroform (2 % crystalline carbolic acid being added in the first two of these changes). It is next treated with benzol until the block clarifies, taken through two changes of soft paraffin (42° C.), into hard paraffin (52° C.) with 5 % beeswax, and embedded in paraffin. Sections are cut at 5–15μ and mounted on albuminized slides. Each slide is immersed in xylol for 5 min., to dissolve the paraffin, then in ether-alcohol (equal parts) for 2–3 min.; the whole slide is next covered with $\frac{1}{2}$ % celloidin and put straightway into 70 % spirit: finally a washing with tap water is given for 1–2 min.

Part II. Silver impregnation. The sections are incubated for 1 hr. at 32° C. in a freshly made solution of protargol (Bayer), made up with tap water and to which, immediately prior to use, has been added 5 g. copper gauze per 100 c.c. They are then rinsed with tap water, and reduced in a solution of 1 g. hydroquinone, 5 g. sodium sulphite, 100 c.c. tap water. The sections, which gradually become tobacco brown in colour, are completely reduced when no further deepening is perceptible—i.e. in 2–3 min. The sections are next washed thoroughly (to remove the hydroquinone) for 3–4 min. in twice-changed tap water, and thereafter covered with a 1 % aqueous solution of gold chloride (containing 3 drops of acetic acid per 100 c.c.) for 5 min.: they become first colourless, later purple. They are again thoroughly washed in twice-changed tap water, and then placed in a 5 % aqueous solution of potassium thyosulphate (hyposulphate) for 5 min. On removal therefrom they are washed and dehydrated, their celloidin coat is removed in ether-alcohol, carbol-xylol and xylol, and the sections are mounted in Canada balsam. Sections of embryo material should remain in the protargol bath for 2 hr.

Drs P. Bacsich and G. M. Wyburn read a paper on "Hormonal analysis of the cyclic variations in the vascular architecture of the guinea-pig uterus", of which the following is an abstract:

In a previous investigation (*J. Anat.* **74**, 139) it was shown that antimesometric hyperaemia can be observed in the uterus of the guinea-pig during

heat, and it was suggested that this cyclic vascular variation has to be included with the other known uterine changes in the histological interpretation of heat in the guinea-pig.

A natural sequel to this work was the endeavour to produce antimesometric hyperaemia in spayed animals by hormonal injection. Thirty spayed animals have been subjected to experiment, and antimesometric hyperaemia has been produced after (1) injections of follicular hormone (oestradiol monobenzoate—Schering), and (2) injections of follicular hormone followed by luteal hormone (proluton—Schering).

Most consistent results were obtained after a preliminary "priming" with small doses of oestrin—possibly an approximation to the physiological dosage.

A characteristic vaginal smear following oestrin treatment is not necessarily accompanied by antimesometric hyperaemia in the uterus, nor does endometrial reaction assure in all cases the presence of the local vascular variation characteristic of normal heat. It is therefore suggested that in an experimentally produced oestrus in the guinea-pig approximation to physiological heat can only be assumed after the successful production of the normal antimesometric hyperaemia.

Professor BOYD inquired concerning the mechanism of the increased uterine vascularization and Professor LE GROS CLARK as to the method of killing employed to retain the blood within the vessels. Professor NICOL thought the suggested growth of blood vessels extremely probable and assigned some role in such growth to the reticulo-endothelial cells. Replying, the authors stated their belief that the antimesometric hyperaemia obtained was paralleled by a concomitant mesometric anaemia: their animals had been gas-killed and the required tissues fixed immediately before blood vessels could empty.

Dr S. ZUCKERMAN and Mr G. I. M. SWYER read a paper on "Post-natal changes in the human prostate", which will be submitted for publication in the *Journal*.

Professor WOOD JONES regarded as mere mucosal vacuoles the histological features interpreted by the authors as incipient new prostatic ducts; he asked how the growth phases of the prostate were determined and was informed that each organ, after careful dissection, was fixed in formol saline and its volume determined. Well preserved material only was employed and shrinkage could be regarded as fairly uniform throughout the series of organs examined. Professor HARRIS stressed the general agreement of the growth curve of the prostrate with that of the female pelvic organs: he commented on the occurrence of prostatic calculi, which were associated with periods of prostatic quiescence, viz. the prepubertal and the involutionary. Professor HAMILTON inquired whether equal shrinkage occurred in the stroma and the parenchyma of the fixed prostates and was informed that, the glandular tissue being more peripherally disposed, any shrinkage of the fibrous tissue of the organ in fixation did not unduly compress the prostate. Dr BACSICH remarked that this paper supported the view that immature tissues resemble adult tissues in their response to stimuli.

Dr E. C. AMOROSO (introduced by W. J. HAMILTON) read a paper on "Tubal transit and rate of cleavage of the dog's ovum", which will be submitted for publication in the *Journal*. The PRESIDENT commented upon the value of this paper, and made comparisons between the histological features

of the dog ovum and that of the cat; the same central cells occurred in both types of ova; the dog material showed no evidence of "enclosure" as was once postulated and in the early dog embryos shown the area of the prochordal plate was already very distinct.

Messrs A. E. Barclay, K. J. Franklin and M. M. L. Prichard read a paper on "A possible mechanism for the protection of the pulmonary alveoli", illustrated by cinematograph, of which the following is an abstract:

Radiographically, insufflated and inhaled radio-opaque dusts can outline the tubular system of the lungs to the termination of the bronchioles. At this point the shadows cease abruptly, and in only a very few instances is there even a suggestion of the presence of dust in the alveoli. The radiographic method, however, illustrates macroscopic conditions only, and does not reveal microscopic quantities.

In three series of experiments, in which three types of dust (lead glass, bismuth carbonate, and uncompressed gas flame carbon) were introduced by (1) inhalation during normal respiration, (2) inhalation during gasping respiration, and (3) insufflation, minute quantities of dust were found, on histological examination, to have entered the alveoli. Although the quantity of dust in the bronchi and bronchioles varied enormously according to the widely divergent methods of introduction (and, to a lesser extent, to the nature of the dust introduced) the amount of dust found in the alveoli was of approximately the same order in all cases. It was microscopic in quantity, in marked contrast to the gross quantities seen in many cases radiographically in the bronchi and bronchioles.

The radiographic and the histological findings are complementary and seem to indicate a defence mechanism protective of the alveoli. This defence mechanism, if existent, is powerless to prevent the entry into the alveoli of introduced fluids and dusts carried by them (Barclay, Franklin & Macbeth, *Brit. J. Radiol.* 1938, N.S. 11, 405–13).

In the subsequent discussion Dr Barclay informed Sir Joseph Barcroft that the introduced dusts actually lined the bronchial walls, Dr Walls that the sphincteric bronchiolar mechanism described was distributed uniformly throughout all parts of the lung, and Professor Le Gros Clark as to details of pharmacological experiments still being pursued in connexion with this mechanism.

Messrs A. E. Barclay, K. J. Franklin and M. M. L. Prichard read a paper on "Notes on the cardio-vascular system of the foetal lamb", which will be submitted for publication in the *Journal*. The communication was commented upon by the President, by Sir Joseph Barcroft and Dr Reagan.

Professor H. A. Harris gave a brief description of certain points illustrated by his demonstration of "The clinical anatomy of the salivary glands".

Dr D. V. Davies spoke briefly on the "Nutrition of the m. extensor pollicis longus in man".

Messrs G. Bourne and S. A. Barnett (introduced by W. E. Le Gros Clark) read a paper on "The distribution of ascorbic acid in chick embryos", of which the following is an abstract:

The distribution of ascorbic acid (vitamin C) has been studied in chick embryos of various ages. This has been made possible by the use of a special

fixative, containing silver nitrate, which gives a black precipitate in the presence of ascorbic acid only. In very early embryos the relation of ascorbic acid to the morphogenetic "tissue movements" has been studied, and in later embryos its relation to the differentiation of various tissues. The method makes possible not only the study of the occurrence of the vitamin in the tissues, but also its intracellular distribution; the quantities present in various cell types have consequently also been investigated.

The PRESIDENT was informed in reply that vitamin C had been observed in the vascular system of 3–4 days old chicks. Mr K. C. RICHARDSON discussed in detail the techniques of fixing and staining employed and suggested that fixation of tissue in liquid air prior to sectioning would yield more satisfactory results.

Drs G. WEDDELL and J. A. HARPMAN read a paper on "Observations on tendon and periosteal pain", of which the following is an abstract:

In human subjects fine, sharp needles were introduced through anaesthetized skin and superficial fascia into the tendon of the biceps brachii muscle, tendo Achillis, and periosteum of the tibia. From peri-tendinous connective tissue and periosteum two types of pain could be aroused. One was mild, sharply localized, and lasted only for the period of the stimulus; the other was more severe, somewhat diffuse, and persisted for some time after the removal of the stimulus. Pain spots are less numerous in peri-tendinous connective tissue and periosteum than in skin.

From the substance of the tendons tested pain could only be aroused from a few spots near the musculo-tendinous junction. This pain was of the mild, brief variety already described.

In methylene blue preparations of human fascia and of rabbit periosteum there occur isolated finer medullated and non-medullated nerve fibres as well as bundles of such fibres bearing free nerve endings. In the substance of tendons from the leg of the rabbit single fibres bearing free nerve endings only occur, disposed in close relation to the neuro-tendinous endings of Golgi and near the musculo-tendinous junction. The fibre in question is non-medullated for the greater part of its course and is related to the Golgi ending in a manner closely resembling that of "accessory" fibres to encapsulated endings. The fibres bearing free endings in tendon undergo characteristic changes in diameter near the "main" ending.

Woollard, Weddell & Harpman (1940) have established the subservience of cutaneous pain by finer medullated and non-medullated fibres bearing free nerve endings. Present findings lend support to the conclusion of Woollard *et al.* that the so-called first (most superficial) type of pain elicited from the human skin is due to stimulation of single units, whilst pain resembling the second (deeper) variety is due to stimulation of bundles of nerve fibres subserving pain.

It is suggested that the "accessory" innervation in question serves to protect "principal" endings from damage due to excessive stimulation.

A paper by the late H. H. WOOLLARD, G. WEDDELL and J. A. HARPMAN on "The anatomical basis for hyperalgesia" was communicated by Dr WEDDELL: it will be submitted for publication in the *Journal*. In the discussion thereon Professor WOOD JONES was informed that nerve fibres had never been traced by the authors higher than the deeper cell layers of the skin and never

therefore into the dying epidermal layers. Accessory fibres were not true collaterals and probably exercised a protective function against over-stimulation. Professor LE GROS CLARK, Professor BOYD and Dr CARLETON also discussed this paper.

Dr R. J. O'CONNOR read a paper on "The role of the pronephros in the development of the nephric system", which will be submitted for publication in the *Journal*.

The PRESIDENT commented in detail upon this paper. He referred to the development of the pronephric duct in elasmobranch fishes and in mammals, in both of which groups pronephric tubules are concerned with pronephric duct formation. He thought that in amphibians also the hinder tubules of the pronephros must play a part in pronephric duct development. Dr O'CONNOR, replying, stressed the development of the duct after extirpation of the pronephros. Professor WOOD JONES drew a distinction between the actual origin of one particular structure from another and the conception of the primary organ exercising control upon the secondarily developing structure: a pronephros, for instance, did not appear essential for the formation of a pronephric duct.

Dr F. P. REAGAN read a paper on "Embryonic and adult sinistral homologues of the vena cava superior in man", an abstract of which follows:

According to certain recent accounts, a portion of vena obliqua atrii sinistri is developed from vena cardinalis anterior sinistra, and a portion of vena hemiazygos is derived from a plexus described by the present author in 1925 as plexus triangularis. Evidence indicates that the accounts referred to are incorrect; they are predicated upon the assumption that the union of sinistral anterior and posterior cardinal veins is closer to right atrium than observable conditions in human embryos indicate; they do not take into account known relationships of lateral mesocardia and roots of lungs; they neglect the fact that the left truncus transversus (left duct of Cuvier) is much longer than the right, and that points of union of anterior and posterior cardinal veins on the same sides continue for some time at practically the same levels, having identical relationships with pulmonary vessels. The acute dorsolateral angle of union between left cardinal vessels often increases to 180° by shifts following degenerative changes in left truncus transversus. The most cranial part of vena cardinalis posterior sinistra (corresponding to pars arciformis of vena azygos) persists completely and receives the inexcusable misnomer of "vena intercostalis suprema". Plexus triangularis initiates by regression the development of saccus lymphaticus jugularis, assisted (in all probability) by a process of extra-intimal replacement. Marshall's classic account is generally confirmed by present work.

Professor J. D. BOYD read a paper on "The early vascularization of the carotid body", which will be submitted for publication in the *Journal*.

This communication was discussed by the PRESIDENT, by Professor LE GROS CLARK and by Dr HAINES. Replying to questions, Professor BOYD said the arterial change-over in the vascular supply of the carotid body conferred upon that body the functional significance of an arterio-venous shunt mechanism, whereby blood could be at one time brought into contact with carotid body cells for their stimulation, or again be directed away from the cells. The carotid body contained some paraganglionic cells (recognizable

from their contained remnants of Nissl granules) as well as mesodermal cells, in direct contact with the blood therein, and derived from the walls of blood vessels.

Professor J. D. BOYD read a paper on "A case of neocerebellar hypoplasia", an abstract of which follows:

The superficial appearance of the brain is described in a dissecting room subject (aged 76) in which there was complete lack of development of the lateral lobes of the cerebellum. The cerebellum is represented by a small nodule only of nervous tissue, presumably palaeocerebellar, and the cerebellar peduncles are much reduced in size. The pons is absent and the pyramidal tracts can be identified on the surface of the ventral aspect of the brain stem as far as their decussation. The olive cannot be definitely identified on the surface. A few transverse fibres cross the pyramids superficially just behind the midbrain. The posterior fossa was well formed and the tentorium normal in position and development. The arachnoid lined the posterior fossa to form an enormous cisterna magna. There are no obvious changes in the size or fissuration of the cerebral cortex.

Clinically there was a right external strabismus, an unsteady gait, whilst articulation was abnormal, slow and slurring.

Professor LE GROS CLARK commented upon the remarkable absence of the pons, particularly the pretrigeminal pontine area, and suggested a histological search for evidence of atrophy and gliosis of the medial thalamic nucleus: he referred to congenital absence of the cerebellum in cats and to its familial incidence in Man.

Professor W. E. LE GROS CLARK read a paper on "The neural and vascular relations of the pineal gland", which will be submitted for publication in the *Journal*.

The subsequent discussion centred mainly upon the nature and significance of the curious arachnoidal tuft described in the paper as projecting from the cerebellum into the floor of the straight sinus. Professor HARRIS described the vascular relationships of the developing human pineal organ, which appeared to grow out into a vascular plexus: he discussed the possibility of the existence of a pineal portal venous system. Professor BOYD, who was informed that arterioles had not been traced into the vascular tuft, discussed the likelihood of the tuft acting as an arterio-venous shunt. Dr BACSICH and Dr HAINES commented upon the histological structure of the arachnoidal tuft. The PRESIDENT was informed upon inquiry that the tuft was not a herniation of the tela choroidea but an extremely localized arachnoidal structure: Professor WOOD JONES was informed that the tuft was lacking in the monkey brains studied. Dr CAVE was answered that the fine filaments from the recurrent ophthalmic nerve of Henle had not been observed in the present investigation.

Professor W. E. LE GROS CLARK read a paper on "Neuronal differentiation in implanted foetal cortical tissue", which will be submitted for publication.

Professor BOYD asked how long the implanted cortical tissue persisted and was told that implants were found to be well vascularized and undergoing progressive development four weeks after operation.

A paper on "The angioarchitecture of the thalamus in the rabbit" by B. SCHLESINGER (introduced by W. E. LE GROS CLARK) was taken as read.

Dr J. A. Harpman read a paper by the late H. H. Woollard, G. Weddell and J. A. Harpman on "The nerve endings subserving warmth", of which the following is an abstract:

Warmth spots were identified in human subjects by means of electrically heated platinum loops 1 mm. in diameter. The introduction of fine, sharp needles into spots so identified frequently arouses a sensation of warmth. On the volar aspect of the forearm the needle has to be introduced to depths of from 1·75 to 2·5 mm.

Areas of skin in which warmth spots were found were excised after the spots had been tattooed with Indian ink.

The excised tissues were impregnated by various reduced silver methods. The spots from which warmth was aroused constantly lay close to sebaceous glands and hair follicles.

A specific type of nerve ending was found in this position. It is rudimentarily encapsulated, consists of loose coils of nerve fibres ending in bulbs, and receives several—up to three—moderately thick nerve fibres. This type of nerve ending somewhat resembles the Ruffini pressure ending; the latter, however, lies deeper in the dermis and is more elongated.

The findings are in agreement with the doctrine of specific nervous activity. They lend further support to the "three-dimensional theory of punctate sensibility" enunciated by Woollard, Weddell & Harpman (1940).

Professor W. E. Le Gros Clark inquired as to the precise nature and definition of Merkel's corpuscles—whether the term "corpuscles" indicated the tactile expansion of a nerve-ending or merely an epidermal structure. He asked whether these corpuscles occurred in human skin, and whether the nerve-endings described as subserving warmth were found in non-human animals, and if so, in what relation to hair follicles. Professor Wood Jones asked whether warmth spots had been observed on the dorsum of the terminal phalanges of the fingers. The President asked whether the corpuscles occurred at the same depth in both hairy and hairless regions of the skin. Dr Cave suggested cetacean skin as worth studying in view of its hairlessness and its enormous subcutaneous fatty layer. Dr Harpman replied.

Drs A. J. E. Cave and R. Wheeler Haines read a paper on "The paranasal sinuses of the anthropoid apes", which will be submitted for publication in the *Journal*.

The President and Professor Appleton approved the technique employed: Professor Le Gros Clark considered the authors' findings likely to prove useful in determining the generic status of certain fossil anthropoid types. Professor Harris discussed the arrangement of air sinuses in the orang skull, with special reference to the relationship of the permanent dentition to the size of the maxillary sinus.

Dr R. Wheeler Haines read a paper on "The independence of sesamoid and epiphysial centres of ossification", which will be submitted for publication in the *Journal*.

Mr Richardson asked whether fibro-cartilage occurred in the region of the attachment to bone of a tendon and was answered in the affirmative. Professor Kirk discussed the ossification of the patella and Dr Carleton homologies of ossificatory patterns in monotremes and marsupials: Dr Romanes inquired concerning the phylogenetic appearance and history of true epiphyses. Mr

RICHARDSON asked concerning the origin of the osteoblasts necessary for the transformation of tendon into bone. Mr CAPENER referred to clinical cases of ectopic bone formation (e.g. in abdominal operation scars) as showing ossification to be independent of pre-existent osseous tissue. Dr HAINES replied, with blackboard demonstrations, to the various questions asked.

Mr N. CAPENER read a paper on "Spondylolysis and spondylolisthesis", of which the following is an abstract:

These closely related conditions, present in 5% of individuals, provide material of great interest alike to the anatomist and to the orthopaedic surgeon. A brief historical review of the subject is presented. Though the embryological evidence is scanty there can be little doubt that lysis is the result of a variation in normal ossification of the posterior neural arch, that in some families it is hereditary, and that not only *lysis* but *-listhesis* is often present at an extremely early age, long before the usual traumatic influences come into play. Spondylolisthesis is well developed before adolescence and its progression in adult life is practically unknown. The dynamics of the lumbo-sacral region are discussed—the various stresses and strains, weight transmission, types of movement, and their influence upon related tissues. A lesion of the intervertebral disk is a likely cause of symptoms which occur in relatively few individuals manifesting this condition. The bearing of these facts upon treatment and operations to prevent sliding are discussed. Such operations are both hazardous and unnecessary.

This communication was discussed by Professors APPLETON, HARRIS and KIRK.

Mr S. WILSON CHARLES read a paper on "Development of the maxilla and mandible and their associated tooth germs", which will be submitted for publication in the *Journal*.

DEMONSTRATIONS

At this Meeting of the Society the following Demonstrations were given:

(1) H. A. HARRIS: Clinical anatomy of the salivary glands.

(2) H. A. HARRIS: Pineal gland region in a series of human embryos.

(3) A. J. E. CAVE: Orbital anomalies in *Colobus badius preussi*.

(4) A. J. E. CAVE and R. W. HAINES: Specimens of anthropoid paranasal sinuses.

(5) D. V. DAVIES: Nutrition of m. extensor pollicis longus in man.

(6) G. J. ROMANES: The cell columns of the spinal cord.

(7) F. P. REAGAN: Developing vascular system, including that of a completely injected 15 mm. human embryo.

(8) A. E. BARCLAY and K. J. FRANKLIN: Cardio-vascular system of foetal lamb.

(9) A. E. BARCLAY and K. J. FRANKLIN: Radiographs of cat lungs, showing distribution of radio-opaque dusts, variously introduced.

REVIEW

The Pineal Organ. By REGINALD J. GLADSTONE and CECIL P. G. WAKELEY. (London: Baillière, Tindall and Cox.) 1940. Price 42*s*.

British anatomists have not been greatly given to the production of exhaustive monographs upon very restricted or even obscure systems of the animal body. There are but few anatomical monographs of British origin that could be cited, if by the definition we assume a monograph to be a separate publication dealing exhaustively with some definite and circumscribed organ or system. It is therefore somewhat of an event that the publication of a volume devoted ostensibly to the Pineal Organ should have followed so closely on the heels of Wilfred Harris's "Brachial Plexus". It would, at first sight, seem almost beyond the range of human erudition to write a supra octavo volume of more than 500 pages, illustrated by more than 300 figures, on the very limited subject of the pineal organ. It is here that the subsidiary title of the book claims attention, for part of it reads "The comparative anatomy of median and lateral eyes, with special reference to the origin of the Pineal Body." It is with this question of the comparative anatomy of median and lateral eyes that the book—from the point of view of space—is mostly concerned. Unfortunately, there does not seem to be any clear indication of the precise manner in which the comparative anatomy of median and lateral eyes is regarded as throwing light upon the origin of the human pineal body. The doubt in the reader's mind is initiated by the reading of the foreword by Sir Arthur Keith. Here we have the statement that the author of the anatomical section of the monograph "had to go back to an early stage of the world of life, one some 400 millions of years removed from us, when median as well as lateral eyes had appeared on the heads of our invertebrate ancestry". Such a statement at once raises the question as to the origin of the vertebrates. Have we, in fact, an invertebrate coelomate ancestry? Unfortunately, Dr Gladstone does not make a very definite pronouncement upon this somewhat essential point. After reading the matter on pages 9 and 136, it is difficult to decide if the vertebrates are postulated as having arisen from the higher invertebrates or if, as in the phylogenetic tree in Fig. 323, the two stocks are presumed to have arisen in complete independence from a primitive coelenterate ancestor. The most definite statement to which the authors appear to have committed themselves occurs on p. 478 in the sentence: "We do not, however, wish it to be inferred that we consider the parietal sense organ of vertebrates has arisen quite independently of the median eyes of invertebrates." Probably no better enterprise was ever undertaken by this Society than that by which it placed its pages at the disposal of Walter Holbrook Gaskell when no other scientific journal would give space to his theories of the origin of the vertebrates. But despite the magic and the apparently irrefutable wealth of detail with which Gaskell, and later Patten, supported the thesis of the origin of the vertebrates from the higher invertebrates, the thesis must be regarded as, at the least, unproven: and the somewhat neglected work of Richard Assheton makes even this verdict a very lenient one. Should it be agreed that the two divergent stocks of the vertebrates and invertebrates arose from some coelenterate ancestor, then much that is included in this volume as implied phylogeny of the vertebrate pineal body must be regarded as no more than interesting collateral evidence of somewhat parallel evolution in the two phyla. Regarded from this point of view the juxtaposition of the accounts of the fusion of the lateral eyes of water-fleas with a description of the condition of cyclopia in the human foetus must be regarded as being somewhat misleading—unless the conditions of parallelism as distinct from phylogeny are made abundantly clear at the outset. More and more, anatomists are being driven to a realization of the fact that in some directions *Homo*

is a very primitive mammalian type. The thesis is in many ways an attractive one; but surely more explanation should have been given in support of the statement that it is no longer so difficult to accept the hypothesis that the parietal foramina of the human skull are remnants of the pineal foramen of lower vertebrates. An appeal to the evidence provided by the therapsid reptiles can hardly be considered as a substitute for some explanation of the strangeness of such a finding. If we omit this consideration—that no clear indication is given as to how the reader is supposed to regard the vast mass of assembled facts—then we must agree wholeheartedly that we are greatly indebted to the authors for the assemblage of these documented statements as to the anatomy of paired and median eyes in the animal series. The volume is a permanent record of the bibliography and the knowledge of all the details relating to the subject and as such is a very welcome contribution to anatomical literature and a welcome addition to the comparatively select list of British anatomical monographs.

To descend to details of criticism, the views put forward on p. 47 would seem to imply very definitely a belief in the inheritance of acquired characters. The reviewer would be loath to question the validity of this belief, but it would seem that the example cited in evidence is a very vulnerable one. In regard to zoological specific names there is a wide variation in the use of capitals: in some cases the specific name is spelled with a capital (mostly in the legends of figures) and in some, lower case is rightly employed. Specific names when translated into English are not always fortunate and "green gull" (p. 75) cannot be considered as a happy substitute for the more usual name of *L. glaucus*. With the clinical aspect of pineal tumours, etc., the reviewer is not so immediately concerned: but at least he can welcome the intimate collaboration of a distinguished surgeon and a distinguished anatomist in the production of an outstanding monograph. Such criticisms as have been made are those that the importance of such a work demands; and they have been made as an outcome of the belief that a monograph that involves wide biological implications should lay down more definite guidance concerning the principles underlying its main thesis.

F. W. J.

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INDEX TO VOLUME LXXIV

Accessory nerve in the sheep. By G. J. Romanes, 336
Adams, W. E. The cervical region of the Lacertilia, 57
Amphibian cloaca, experimental study of the development. By R. J. O'Connor, 301
— mesonephros, experiments on the development. By R. J. O'Connor, 34
Anthropoid apes, paranasal sinuses. By A. J. E. Cave and R. W. Haines, 493
Ascorbic acid, distribution in chick embryos. By G. Bourne and S. A. Barnett, 554
Australian aboriginal skulls, fronto-palatine suture. By K. F. Russell, 129
Australopithecus, a new reconstruction. By A. J. E. Cave, 139

Bacsich, P. Some observations on the Bodian silver method with an account of a new modification, 552
— and Wyburn, G. M. Hormonal analysis of the cyclic variations in the vascular architecture of the guinea-pig uterus, 552
Barclay, A. E., Franklin, K. J. and Prichard, M. M. L. A possible mechanism for protection of the pulmonary alveoli, 554
Barnett, S. A. *See* Bourne, G., joint authors, 554
Barr, Murray L. Observations on the morphology of the synapse in the cat's spinal cord, 1
Basilar membrane, length in man and in various mammals. By J. A. Keen, 524
Blair, D. M. and Davies, F. The conducting system in the human and in the calf heart, 137
Bodian silver method, a new modification. By P. Bacsich, 552
Bone, evolution of Haversian pattern. By G. N. C. Crawford, 284
Bourne, G. and Barnett, S. A. The distribution of ascorbic acid in chick embryos, 554
Boyd, J. D. A case of neocerebellar hypoplasia, 557
Brash, J. C. Vital staining of bone with hydroxyanthraquinone derivatives, 141
Brigg, E. A. Anatomy of the Sheep's Brain. Review, 411
Brocklehurst, R. J. and Edgeworth, F. H. The fibre components of the laryngeal nerves of *Macaca mulatta*, 386

Capener, N. Spondylolysis and spondylolisthesis, 559
Cave, A. J. E. A new reconstruction of *Australopithecus*, 139
— and Haines, R. W. The paranasal sinuses of the anthropoid apes, 493
Cephalomelus: a rare tumour in a lamb. By D. V. Davies, 403
Cerebellum and pons, projection of the cerebral cortex. By S. Sunderland, 201
Cervical region of the Lacertilia. By W. E. Adams, 57
Clark, W. E. Le Gros. The nervous and vascular relations of the pineal gland, 471
— The Tissues of the Body. Review, 144
Cloaca, amphibian, an experimental study of the development. By R. J. O'Connor, 301
Colliculus, inferior, connexions. By H. H. Woollard and J. A. Harpman, 441
Conducting system in the human and in the calf heart. By D. M. Blair and F. Davies, 137
— — of the heart of the salamander. By F. Davies and E. T. B. Francis, 138
Congenital absence of one leg, the central nervous system. By W. Walls, 141
Cooper, E. R. A. Human Histology, a guide for medical students. Review, 411
Corpus callosum and hippocampus, relative position in placental mammals. By F. Goldby, 227
Cortex, cerebral, projection on the pons and cerebellum. By S. Sunderland, 201
Corticofugal degeneration following thermocoagulation of areas 4, 6, and 4-s in *Macaca mulatta*. By W. J. C. Verhaart and M. A. Kennard, 239
Crawford, G. N. C. The evolution of the Haversian pattern in bone, 284
Cutaneous pain, neurohistological basis. By H. H. Woollard, G. Weddell and J. A. Harpman, 413

Davies, D. V. Cephalomelus: a rare tumour in a lamb, 403
Davies, F. Mounting media and the fading of histological preparations, 464
— *See* Blair, D. M., joint authors, 137
— and Francis, E. T. B. The conducting system of the heart of the salamander, 138
Diaphragmatic hernia, description of a rare type. By H. D. O'Brien, 131
Dickson, L. M. The development of nerve-endings in the respiratory muscles of the sheep, 268
Drinker, C. K. *See* Yoffey, J. M., joint authors, 45

Ectotympanic, lemurine form in a primitive marsupial. By F. Wood Jones and V. F. Lambert, 72
Edgeworth, F. H. *See* Brocklehurst, R. J., joint authors, 386
Epiphyses in *Sphenodon*. By R. Wheeler Haines 80

Francis, E. T. B. *See* Davies, F., joint authors, 138
Franklin, K. J. *See* Barclay, A. E., joint authors, 554
Frazer, J. E. The Anatomy of the Human Skeleton. Review, 412
Fronto-palatine articulation in aboriginal Australian skulls. By K. F. Russell, 129

Gladstone, R. J. and Wakeley, C. P. G. The Pineal Organ. Review, 560
Goldby, F. An experimental investigation of the motor cortex and its connexions in the phalanger, *Trichosurus vulpecula*, 12
— On the relative position of the hippocampus and the corpus callosum in placental mammals, 227
Gray, J. H. Studies of the regeneration of lymphatic vessels, 309
Groome, J. R. *See* Zuckerman, S., joint authors, 171
Gum of *Talpa europea*, innervation. By W. Lewinsky and D. Stewart, 53
Guttmann, L. The distribution of disturbances of sweat secretion after extirpation of certain sympathetic cervical ganglia in man, 537

Haines, R. Wheeler. The structure of the epiphyses in *Sphenodon* and the primitive forms of secondary centres, 80
— *See* Cave, A. J. E., joint authors, 493
Hallpike, C. S. and Peet, E. W. A modification of the technique of wax-plate construction, 127
Harpman, J. A. *See* Weddell, G., joint authors, 255
— *See* Woollard, H. H., joint authors, 413
— *See* Woollard, H. H., joint authors, 441
— *See* Weddell, G., joint authors, 555
— *See* Woollard, H. H., joint authors, 558
Haversian pattern in bone, evolution. By G. N. C. Crawford, 284
Hippocampus and corpus callosum, relative position in placental mammals. By F. Goldby, 227
Histological preparations, fading, and mounting media. By F. Davies, 464

In Memoriam. Robert William Reid, 406
Ince, J. G. Hastings. *See* Young, M., joint authors, 369
— *See* Young, M., joint authors, 374
Innervation of the musculature of the tongue. By G. Weddell, J. A. Harpman, D. G. Lambley and L. Young, 255
Intersexuality, experimental study of the morphogenesis. By S. Zuckerman and J. R. Groome, 171
Intestine, small, intramural nervous system. By Pei-Lin Li, 348

Johnston, T. B. A Synopsis of Regional Anatomy. Review, 144
Joints, synovial, development. By J. Whillis, 277

Keen, J. A. A note on the length of the basilar membrane in man and in various mammals, 524
Kennard, M. A. *See* Verhaart, W. J. C., joint authors, 239

Lacertilia, cervical region. By W. E. Adams, 57
Lambert, V. F. *See* Wood Jones, F., joint authors, 72
Lambley, D. G. *See* Weddell, G., joint authors, 255
Laryngeal nerves of *Macaca mulatta*. By R. J. Brocklehurst and F. H. Edgeworth, 386
Lemniscus, lateral, connexions. By H. H. Woollard and J. A. Harpman, 441
Lemurine form of the ectotympanic in a primitive marsupial. By F. Wood Jones and V. F. Lambert, 72
Lewinsky, W. and Stewart, D. The innervation of the gum of *Talpa europea*, 53
Lightoller, G. S. The comparative morphology of the M. caninus, 397
— The comparative morphology of the platysma, 390
Lumbosacral region of the human spine, transmutation of vertebrae. By M. Young and J. G. Hastings Ince, 369
Lymphatic vessels, studies of the regeneration. By J. H. Gray, 309
Lymphatics of the nasal mucous membrane in the cat and monkey. By J. M. Yoffey and C. K. Drinker, 45

McGregor, A. L. Synopsis of Surgical Anatomy. Review, 146
Mamillary body in mammals and man. By Jerzy Rose, 91
Martin, C. P. and O'Brien, H. D. Movements at the shoulder joint, 140
Mesonephros, amphibian, experiments on the development. By R. J. O'Connor, 34
Metacarpal and metatarsal bones in children, radiographic studies. By K. Posener, E. Walker and G. Weddell, 76
Models, blotting-paper, construction. By R. L. de C. H. Saunders, 406
Motor cortex in the phalanger, *Trichosurus vulpecula*. By F. Goldby, 12
Mounting media and the fading of histological preparations. By F. Davies, 464
Musculus caninus, comparative morphology. By G. S. Lightoller, 397

Nasal mucous membrane in the cat and monkey, lymphatics. By J. M. Yoffey and C. K. Drinker, 45
Neocerebellar hypoplasia. By J. D. Boyd, 557
Nerve, spinal accessory in the sheep. By G. J. Romanes, 336
Nerve-endings in respiratory muscles, development. By L. M. Dickson, 268
Nerves, laryngeal, in *Macaca mulatta*. By R. J. Brocklehurst and F. H. Edgeworth, 386
Neurohistological basis of cutaneous pain. By H. H. Woollard, G. Weddell and J. A. Harpman, 413

O'Brien, H. D. Pericardio-peritoneal communication. Description of a rare type of diaphragmatic hernia, 131
— *See* Martin, C. P., joint authors, 140
O'Connor, R. J. Experiments on the development of the amphibian mesonephros, 34
— An experimental study of the development of the amphibian cloaca, 301

Pain, tendon and periosteal. By G. Weddell and J. A. Harpman, 555

Palate, the nature of the soft. By F. Wood Jones, 147
Paranasal sinuses of the anthropoid apes. By A. J. E. Cave and R. W. Haines, 493
Patella, development. By R. Walmsley, 360
Peet, E. W. *See* Hallpike, C. S., joint authors, 127
Pei-Lin Li. The intramural nervous system of the small intestine, 348
Pelvis, male and female, radiographic comparison. By M. Young and J. G. Hastings Ince, 374
Pericardio-peritoneal communication. By H. D. O'Brien, 131
Phalanger, motor cortex and its connexions. By F. Goldby, 12
Pineal gland, nervous and vascular relations. By W. E. Le Gros Clark, 471
Pituicytes in the human foetus. By W. M. Shanklin, 459
Platysma, comparative morphology. By G. S. Lightoller, 390
Pons and cerebellum, projection of cerebral cortex. By S. Sunderland, 201
Posener, K., Walker, E., and Weddell, G. Radiographic studies of the metacarpal and metatarsal bones in children, 76
Prichard, M. M. L. *See* Barclay, A. E., joint authors, 554
Pulmonary alveoli, a possible mechanism for the protection. By A. E. Barclay, K. J. Franklin and M. M. L. Prichard, 554

Radiographic comparison of the male and female pelvis. By M. Young and J. G. Hastings Ince, 374
— studies of the metacarpal and metatarsal bones in children. By K. Posener, E. Walker and G. Weddell, 76
Reagan, F. P. Embryonic and adult sinistral homologues of the vena cava superior in man, 556
Rectal and uterine veins, anastomoses. By E. G. Wermuth, 116
Reid, Robert William. In Memoriam, 409
Respiratory muscles, development of nerve endings. By L. M. Dickson, 268
Reviews. Anatomy of the Human Skeleton. By J. Ernest Frazer, 412
— Anatomy of the Sheep's Brain. By E. A. Brigg, 411
— Handbuch d. vergl. Anatomie d. Wirbeltiere, 146
— Human Histology, a guide for medical students. By E. R. A. Cooper, 411
— Pineal Organ, The. By R. J. Gladstone and C. P. G. Wakeley, 560
— Practical Anatomy, Manual. By T. Walmsley, 146
— Principles of Development. By Paul Weiss, 411
— Synopsis of Regional Anatomy. By T. B. Johnston, 144
— Tissues of the Body. By W. E. Le Gros Clark, 144
Russell, K. F. The presence of a fronto-palatine articulation in aboriginal Australian skulls, 129

Saunders, R. L. de C. H. Notes on the construction of blotting-paper models, 406
Schlesinger, B. The venous drainage of the brain with special reference to the Galenic system, 142
Shanklin, W. M. Differentiation of pituicytes in the human foetus, 459
Sphenodon, epiphyses and the primitive form of secondary centres. By R. Wheeler Haines, 80
Spinal cord, morphology of the synapse. By Murray L. Barr, 1
Spondylolysis and spondylolisthesis. By N. Capener, 559
Stewart, D. *See* Lewinsky, W., joint authors, 53
Sunderland, S. The projection of the cerebral cortex on the pons and cerebellum in the macaque monkey, 201
Sweat secretion, disturbances after extirpation of sympathetic cervical ganglia. By L. Guttmann, 537
Sympathetic cervical ganglia, extirpation, and disturbances of sweat secretion. By L. Guttmann, 537
Synapse, morphology in the cat's spinal cord. By Murray L. Barr, 1
Synovial joints, development. By J. Whillis, 277

Talpa europea, innervation of the gum. By W. Lewinsky and D. Stewart, 53
Temporal lesions and thalamic degeneration in the cat. By W. H. Waller, 528
Thalamic degeneration induced by temporal lesions in the cat. By W. H. Waller, 528
Tongue musculature, innervation. By G. Weddell, J. A. Harpman, D. G. Lambley and L. Young, 255

Uterine and rectal veins, anastomoses. By E. G. Wermuth, 116
Uterus, hormonal analysis of the cyclic variations in the vascular architecture in the guinea-pig. By P. Bacsich and G. M. Wyburn, 552

Vena cava superior in man, sinistral homologues. By F. P. Reagan, 556
Venous drainage of the brain. By B. Schlesinger, 142
Verhaart, W. J. C. and Kennard, M. A. Corticofugal degeneration following thermocoagulation of areas 4, 6 and 4-s in *Macaca mulatta*, 239
Vertebrae, transmutation in the lumbo-sacral region of the human spine. By M. Young and J. G. Hastings Ince, 369
Vital staining of bone with hydroxyanthraquinone derivatives. By J. C. Brash, 141

Wakeley, C. P. G. *See* Gladstone, R. J., joint authors, 560
Walker, E. *See* Posener, K., joint authors, 76
Waller, W. H. Thalamic degeneration induced by temporal lesions in the cat, 528
Walls, W. The central nervous system in a case of congenital absence of one leg, 141
Walmsley, R. The development of the patella, 360

Walmsley, T. Manual of Practical Anatomy. Review, 146
Warmth, the nerve endings subserving. By H. H. Woollard, G. Weddell and J. A. Harpman, 558
Wax plate construction, a modification of the technique. By C. S. Hallpike and E. W. Peet, 127
Weddell, G. and Harpman, J. A. Observations on tendon and periosteal pain, 555
— — Lambley, D. G. and Young, L. The innervation of the musculature of the tongue, 255
— *See* Posener, K., joint authors, 76
— *See* Woollard, H. H., joint authors, 413
— *See* Woollard, H. H., joint authors, 558
Weiss, P. Principles of Development. Review, 411
Wermuth, E. G. Anastomoses between the rectal and uterine veins, 116
Whillis, J. The development of synovial joints, 277
Wood Jones, F. The nature of the soft palate, 147
— and Lambert, V. F. The occurrence of the lemurine form of the ectotympanic in a primitive marsupial, 72
Woollard, H. H. and Harpman, J. A. The connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus, 441
— Weddell, G., and Harpman, J. A. Observations on the neurohistological basis of cutaneous pain, 413
— — — The nerve endings subserving warmth, 558
Wyburn, G. M. *See* Bacsich, P., joint authors, 552

Yoffey, J. M. and Drinker, C. K. Some observations on the lymphatics of the nasal mucous membrane in the cat and monkey, 45
Young, L. *See* Weddell, G., joint authors, 255
Young, M. and Hastings Ince J. C. A radiographic comparison of the male and female pelvis, 374
— — Transmutation of vertebrae in the lumbosacral region of the human spine, 369

Zuckerman, S. and Groome, J. R. An experimental study of the morphogenesis of intersexuality, 171

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CONTENTS. I. On Generalized Analysis of Variance (1). By P. L. Hsu. II. The Derivation of the Fifth and Sixth Moments of the Distribution of s, in Samples from a Normal Population. By C. T. Hsu and D. N. Lawley. III. Testing the Homogeneity of a Set of Variances. By H. O. Hartley. IV. The Simultaneous Distribution in Samples of Mean and Standard Deviation, and of Mean and Variance. By L. Trukša. V. Certain Projective Depth and Breadth Measurements of the Facial Skeleton in Man. By A. Schreiner. VI. Transposition of the Viscera and other Reversals of Symmetry in Monozygotic Twins. By E. A. Cockayne. VII. The Human Remains of the Iron Age and Other Periods from Maiden Castle, Dorset. By C. N. Goodman and G. M. Morant. VIII. Homogeneity of Results in Testing Samples from Poisson Series. With an Application to Testing Clover Seed for Dodder. By J. Przyborowski and H. Wilenski. IX. On the Method of Paired Comparisons. By M. G. Kendall and B. Babington Smith. X. The Mean and Variance of χ^2, when used as a Test of Homogeneity, when Expectations are Small. By J. B. S. Haldane. XI. A Note on the Statistical Analysis of Sentence-length as a Criterion of Literary Style. By C. B. Williams. XII. Applications of the non-central t-distribution. By N. L. Johnson and B. L. Welch.

Miscellanea. (i) Stirling's Formula with Remainder. By E. V. Huntington. (ii) A Note on the Interpretation of Quasi-sufficiency. By M. S. Bartlett. (iii) The Cumulants and Moments of the Binomial Distribution, and the Cumulants of χ^2 for a $(n \times 2)$-fold Table. By J. B. S. Haldane. (iv) Review of A. A. Tschuprow's *The Principles of the Mathematical Theory of Correlation.*

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CONTENTS OF PART IV

VOLUME LXXIV

PAGE

The late H. H. WOOLLARD, GRAHAM WEDDELL and J. A. HARPMAN. Observations on the neurohistological basis of cutaneous pain (with 13 Text-figures and Plates I to IV) 413

The late H. H. WOOLLARD and J. A. HARPMAN. The connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus (with 2 Text-figures and Plates I and II) 441

WILLIAM M. SHANKLIN. Differentiation of plasmocytes in the human foetus (with 2 Text-figures and Plates I and II) 459

F. DAVIES. Mounting media and the fading of histological preparations 464

W. E. LE GROS CLARK. The nervous and vascular relations of the pineal gland (with 8 Text-figures and Plates I and II) 471

A. J. E. CAVE and R. WHEELER HAINES. The parasinal sinuses of the anthropoid apes (with 21 Text-figures) 493

J. A. KEEN. A note on the length of the basilar membrane in man and in various mammals (with 2 Text-figures) 524

W. H. WALLER. Thalamic degeneration induced by temporal lesions in the cat (with 5 Text-figures) 528

L. GUTTMAN. The distribution of disturbances of sweat secretion after extirpation of certain sympathetic cervical ganglia in man (with 9 Text-figures) 537

PROCEEDINGS 552

REVIEW: *The Pineal Organ*. By REGINALD J. GLADSTONE and CECIL P. G. WAKELEY 560

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